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The Limited Oxidation of Cellulose with Nitrogen Dioxide in Carbon Tetrachloride

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THE LIMITED OXIDATION OF CELLULOSE WITH NITROGEN DIOXIDE IN CARBON TETRACHLORIDE

A thesis submitted by

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INTRODUCTION

Since the discovery that nitrogen dioxide* oxidized cellulose (1), this reagent has been investigated with interest. Cellulose has been oxidized with gaseous nitrogen dioxide by the static method (2, 4) in which the cellulose is suspended in an atmosphere of nitrogen dioxide in a closed vessel, and by the cyclic method (2, 3) in which the cellulose is placed in a vessel through which gaseous nitrogen dioxide is circulated. Cellulose has also been oxidized with a solution of nitrogen dioxide in carbon tetrachloride (5, 6).

The predominant attack on cellulose by nitrogen dioxide is the oxidation of primary hydroxyl groups of anhydroglucose units to carboxyl groups (2, 3, 7, 8, 9). Carboxyl contents as high as 20% by weight are easily attainable, and the theoretical maximum of 25.6%, at which all primary hydroxyl groups have been oxidized to carboxyl groups, can be exceeded (3, 4). In order to account for carboxyl contents greater than the theoretical maximum, other locations on the anhydroglucose units must be subject to attack. Side reactions resulting in the formation of carbonyl groups have been reported (4, 8, 10, 11, 12). The properties of oxidized celluloses prepared by either gaseous nitrogen dioxide or nitrogen dioxide or nitrogen dioxide-carbon tetrachloride solutions appear to be the same at equal carboxyl contents.

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*Nitrogen dioxide will be used to designate the equilibrium mixture of nitrogen dioxide and nitrogen tetroxide existing under the conditions of the oxidation experiments.

In this paper, the term "celluronic acid (s)" will be used to designate the products obtained from the nitrogen dioxide oxidation of cellulose. For simplification, any product resulting from this oxidation, regardless of carboxyl content, will be referred to as a "celluronic acid." The application of this term to the nitrogen dioxide oxidation products is suitable since true "celluronic acids", i.e., polyanhydroglucuronic acids, are not found in nature.

Even at high degrees of oxidation (carboxyl contents of 7 to 32%, which correspond to oxygen consumptions of 0.6 to 2.9 atom per anhydroglucose unit), celluronic acids are fluffy, white materials which retain the fibrous appearance of the original cellulose, and cannot be visually distinguished from it (2,4). This fibrous nature is in contrast to cellulose oxidized by chromic acid which is powdery and friable at an oxygen consumption of 0.4 atom per anhydroglucose unit. Chromic acid oxidizes only the amorphous regions of the cellulose (13). Friable products similar to chromic acid-oxidized cellulose are also obtained by over bleaching with hypohalites (14), peroxides, and permanganate. The celluronic acids are also distinguished from these other oxidized celluloses by their retention of as much as 20% of the original tensile strength at carboxyl contents corresponding to an oxygen consumption of 0.6 atom per anhydroglucose unit* (4). The tensile strength of yarns oxidized with chromic acid decreases to zero at oxygen consumptions greater than 0.4 atom per anhydroglucose unit (13).

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* An oxygen consumption of 0.6 atom per anhydroglucose unit corresponds to 7% carboxyl. Commercial specifications for oxidized gauze require a retention of 40% tensile after a carboxyl content of 16% is introduced.

X-ray diagrams offer still another means for distinguishing celluronic acids from celluloses oxidized with chromic acid (13) and similar oxidants (14). In the latter, the x-ray diffraction patterns remain sharp and do not change with increasing degrees of oxidation, whereas those of celluronic acids prepared by gaseous oxidations gradually become more diffuse after 50% of the anhydroglucose units have been oxidized (15). By the time the theoretical maximum number of carboxyl groups have been introduced, the x-ray diagrams of the celluronic acids no longer resemble the diagram of cellulose.

X-ray diffraction patterns also indicated that the crystallites of cotton linters were completely swollen in a 50% solution of nitrogen dioxide in carbon tetrachloride before significant oxidation occurred (16). These diffraction patterns were offered as proof that the swelling power of the nitrogen dioxide-carbon tetrachloride solution is the factor which controls the location and rate of oxidation.

Sufficient carboxyl groups can be introduced into celluronic acids to render them soluble in dilute alkalies (1,2,10). For example, celluronic acids with greater than 9.0% carboxyl are soluble in 0.1 N sodium hydroxide at 20°C. (4). With lower carboxyl contents, they are partially soluble.

The solubility of high carboxyl celluronic acids in body fluids (17), and their hemostatic properties (18) make them applicable to commercial utilization. Celluronic acids are now being produced commercially (19) for use in surgical dressings in the form of bulk packing or gauze dressings. Gauze specifications require that the oxidation

introduce 16% carboxyl while 40% of the original tensile strength is maintained (19).

Other noteworthy properties of celluronic acids include the insolubility of nitrated celluronic acids in acetone (4) and the high affinity of celluronic acids for water (4). With moisture adsorption ratios* as high as 4.0, celluronic acids of high carboxyl content are the only known type of acidic-oxidized cellulose with so great an affinity for water.

The retention of the fibrous nature and tensile strength in celluronic acids are properties which are regarded as indexes of relatively high D.P. Retention of molecular length would be anticipated if only primary hydroxyl groups were oxidized to carboxyl groups. Although evidence points to the simple oxidation of primary hydroxyl groups to carboxyl groups as being most prominent, side reactions involving the formation of alkali-sensitive carbonyl groups are used to explain the reduction in chain length implied by the low relative viscosities of celluronic acids in alkaline solvents (4,8,10) and the loss of material on regeneration of celluronic acids from alkali (4).

At higher degrees of oxidation, primary hydroxyl groups all along the polyanhydroglucose chains are oxidized, and carbonyl groups would be expected to be similarly distributed along the chains. However, as oxidation proceeds, the relative viscosities of the celluronic acids

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* Moisture adsorption ratio is the ratio of the amount of water adsorbed by oxidized cellulose to that adsorbed by unmodified cellulose under similar conditions.

rapidly reach a leveling-off value at a magnitude corresponding to the D.P. of crystallites--a characteristic of celluloses which have been degraded in the accessible regions. If alkali-sensitive carbonyl groups were introduced all along the chains, the alkaline D.P. would continue to decrease the values below the D.P. of crystallites.

Side reactions introducing alkali-sensitive groups into the celluronic acid molecules probably involve the oxidation of carbon atoms 2 or 3 of the anhydroglucose units to carbonyl groups. In view of the current knowledge of the alkali sensitivity of other types of oxidized celluloses (20-22), these carbonyl groups could be responsible for chain scission in alkaline solvents. The products of a chain scission in alkali would be two smaller celluronic acid molecules and a complex mixture of degraded products. The formation of additional acidic groups by alkaline degradation has been demonstrated by alkali consumptions which increase as the time of reaction with excess alkali is prolonged (2,4,10).

The presence of reducing groups in celluronic acids has been demonstrated by high copper numbers (3,4,8,10,23). The formation of reducing groups can be the result of chain scission in the alkaline copper reagent (4,8,10). Carbonyl groups have also been found in celluronic acids* by the methylhydroxylamine method (10) and adsorption spectra (11). The presence of aldehyde groups has been detected by measuring the increase in carboxyl content and decrease in copper

* In a recent investigation (61), carbonyl groups in celluronic acids have been detected by their reaction with hydroxylamine hydrochloride and sodium borohydride.

number on oxidation of celluronic acids with chlorous acid (4). The carbonyl groups found could be terminal aldehyde groups (carbon 1) as well as aldehyde groups on carbons 2, 3, or 6, or ketone groups on carbons 2 or 3.

The oxidation of hydroxyl groups on carbons 2 and 3 has been supported by the derivation of a triketo compound, 3,5-dioxotetrahydro-γ-pyrone from a celluronic acid with 13% carboxyl (12). The isolation of this compound indicates that nitrogen dioxide oxidation may be accompanied by a simultaneous oxidation on carbons 2 and 3 to ketone groups on some anhydroglucose units.

Degradation resulting in chain scissions due to acid hydrolysis or oxidation of glycosidic linkages in localized areas could also occur during oxidation, but these degradations have not been used as a basis for explaining the properties of celluronic acids. Conflicting opinions have been expressed as to whether the oxides of nitrogen present in the oxidizing medium will (23) or will not (8) be capable of forming acid concentrations of sufficient strength to cause hydrolysis.

PRESENTATION OF THE PROBLEM

Since celluronic acids retain the fibrous structure and about 20% of the tensile strength of unoxidized cellulose, Nevell (4) interpreted these properties as "evidence that the oxidation of cotton by nitrogen dioxide is not accompanied by a very large reduction in the average length of the chain-molecules." Nevell further anticipated that "the presence of a high proportion of long chain-molecules in the oxycelluloses would be expected to result in their solutions possessing high intrinsic viscosities." Since this was not the case, Nevell stated, "The low relative viscosities are not due to scission of chain-molecules during the preparation of the materials as they are in the case of hydrocelluloses, which fall to powder before a relative viscosity... of 1.6 is reached. They are due to scission resulting from the alkaline nature of the solvent used."

Indeed, as oxidation progresses, the carbonyl content estimated by copper number (4) and methylhydroxylamine hydrochloride (10) increases, and indicates a potential source of chain scission in alkali. The literature has supported this theory of alkali sensitivity.

If alkali sensitivity due to the introduction of carbonyl groups were the sole cause of low D.P., one would expect the higher stages of oxidation to be accompanied by a progressively lower D.P. Contrary to this, cuprammonium relative viscosities reach a relatively constant value which is independent of oxidation (4). Therefore, the writer has found this explanation, i.e., alkali sensitivity, for the properties of celluronic acids to be inadequate. As an alternate hypothesis, it

is proposed that hydrolysis and/or localized oxidations resulting in chain scission occur during nitrogen dioxide oxidation. Through an experimental program which is based on the assumptions that (1) the critical period of oxidation is that period in which the rapid decrease in D.P. occurs, and (2) that the true D.P. of the celluronic acids can be measured in a nonalkaline solvent, some further insight should be gained into the properties of celluronic acids.

The purpose of this thesis is to demonstrate whether or not degradation does occur during oxidation with nitrogen dioxide in carbon tetrachloride and to provide information about the locus of oxidation during the initial stages.

MATERIALS AND METHODS

COTTON LINTERS

The cellulose used for nitrogen dioxide oxidations was mill run cotton linters which had been given a 2% sodium hydroxide cook and a 0.5% chlorine bleach. The linters were obtained in slush form at about 10% consistency. The linters were solvent dried with acetone and ether prior to oxidation. Details of linters preparation may be found in the Appendix.

NITROGEN DIOXIDE OXIDATIONS

PRELIMINARY OXIDATIONS

Two-gram samples of cotton linters were oxidized with nitrogen dioxide-carbon tetrachloride mixtures containing 3, 6, 12, 25, and 50% nitrogen dioxide by weight. The oxidizing medium was prepared and brought to 20°C. prior to addition to 125-ml. glass-stoppered Erlenmeyer flasks containing the cotton linters. The flasks were then placed in a 20°C. water bath for the desired reaction time. During oxidation the flasks were occasionally swirled to insure complete mixing. A weight ratio of oxidation medium to cellulose of 90:1 was used for most experiments, although ratios of 135:1 and 50:1 were also used.

After oxidation, the celluronic acids produced were washed on a fritted glass (C) filter funnel with 200 ml. of carbon tetrachloride followed by 200 ml. of absolute methanol. The celluronic acids were then soaked for five minutes in 50% aqueous methanol and dried by solvent exchange with 200 ml. of acetone followed by 200 ml. of anhydrous ether.

Excess ether was removed by drying the samples until odorless at 50°C. under vacuum.

OXIDATIONS WITH SIX PERCENT NITROGEN DIOXIDE IN CARBON TETRACHLORIDE

Sixty-gram batches of cotton linters were oxidized in 2000-ml. resin reaction flasks equipped with Dow Corning 550 Fluid seals around the shaft of the four-paddle, stainless steel stirrers. The cotton linters were placed in the reaction flask which was then assembled and placed in a water bath at 20°C. The oxidizing medium of 180 grams of nitrogen dioxide in 2820 grams of carbon tetrachloride previously brought to 20°C. was then added to the reaction flask. The agitation supplied by the four-paddle stirrer was sufficient to mix quickly the oxidizing medium and cotton linters into a uniform suspension.

The weight ratio of oxidation medium to cellulose of 50:1 was selected so that the oxidation medium would cover the linters completely. With this ratio, the large excess of nitrogen dioxide present would permit only a small change in solution concentration during oxidation.

After the desired reaction period had elapsed, the apparatus was disassembled and the contents poured onto a 16-cm. fritted glass (C) filter funnel with suction applied. The celluronic acids were then (a) washed on the filter with 400 ml. of carbon tetrachloride followed by two 400-ml. portions of absolute methanol, (b) removed from the filter and soaked for 5 minutes in 50% aqueous methanol, (c) returned to the filter for removal of the aqueous methanol, and (d) dried by

solvent exchange with acetone and ether. Excess ether was removed from the celluronic acids by evacuation in a vacuum desiccator overnight at room temperature. Celluronic acids for analysis were subsequently dried at 50°C. under vacuum until odorless, or for 2 hours, whichever was longer.

The methanol was used in the washing sequence to stop the reaction. Methanol reacts instantaneously with nitrogen dioxide, as noted by the disappearance of the characteristic brown color of nitrogen dioxide.

REAGENTS

The nitrogen dioxide used was a cylinder gas supplied by the Matheson Co. The carbon tetrachloride was reagent grade.

REACTIONS

Details on the preparation of the cotton linters, methods of celluronic acid modification, and methods of analysis may be found in the Appendix.

EXPERIMENTAL RESULTS AND DISCUSSION

PRELIMINARY OXIDATION OF COTTON LINTERS

The data obtained from preliminary oxidation experiments are illustrated in Figures 1 and 2 and tabulated in Table I. All experimental data in table form are located in the Appendix.

For Figure 1, the carboxyl content obtained by oxidation has been plotted against concentrations of nitrogen dioxide in carbon tetrachloride for oxidation times from 0.5 to 24 hours. This figure and the data of Table I demonstrate that the concentration of nitrogen dioxide apparently has the same effect on carboxyl groups formed at all time intervals of oxidation, i.e., about 3 to 4 times as many carboxyl groups are introduced in 50% solution as in 3% solution.

It has been reported (16) that cotton linters oxidized in a 50% solution of nitrogen dioxide in carbon tetrachloride are attacked simultaneously in accessible and crystalline regions. This conclusion was reached after it was found that a straight line fitted the data of logarithm percent anhydroglucose units unoxidized *vs.* time of oxidation. The early stages of oxidation, if studied, were not reported.

The data of Figure 1 have been calculated in terms of anhydroglucose units unoxidized and plotted as logarithm *vs.* time of oxidation in Figure 2. If a first order reaction occurs, the curves of Figure 2 should be straight lines. Even at 50% concentration of nitrogen dioxide, a straight line is not obtained on oxidation for 24 hours and the reaction is not first order throughout this interval. This is contrary to

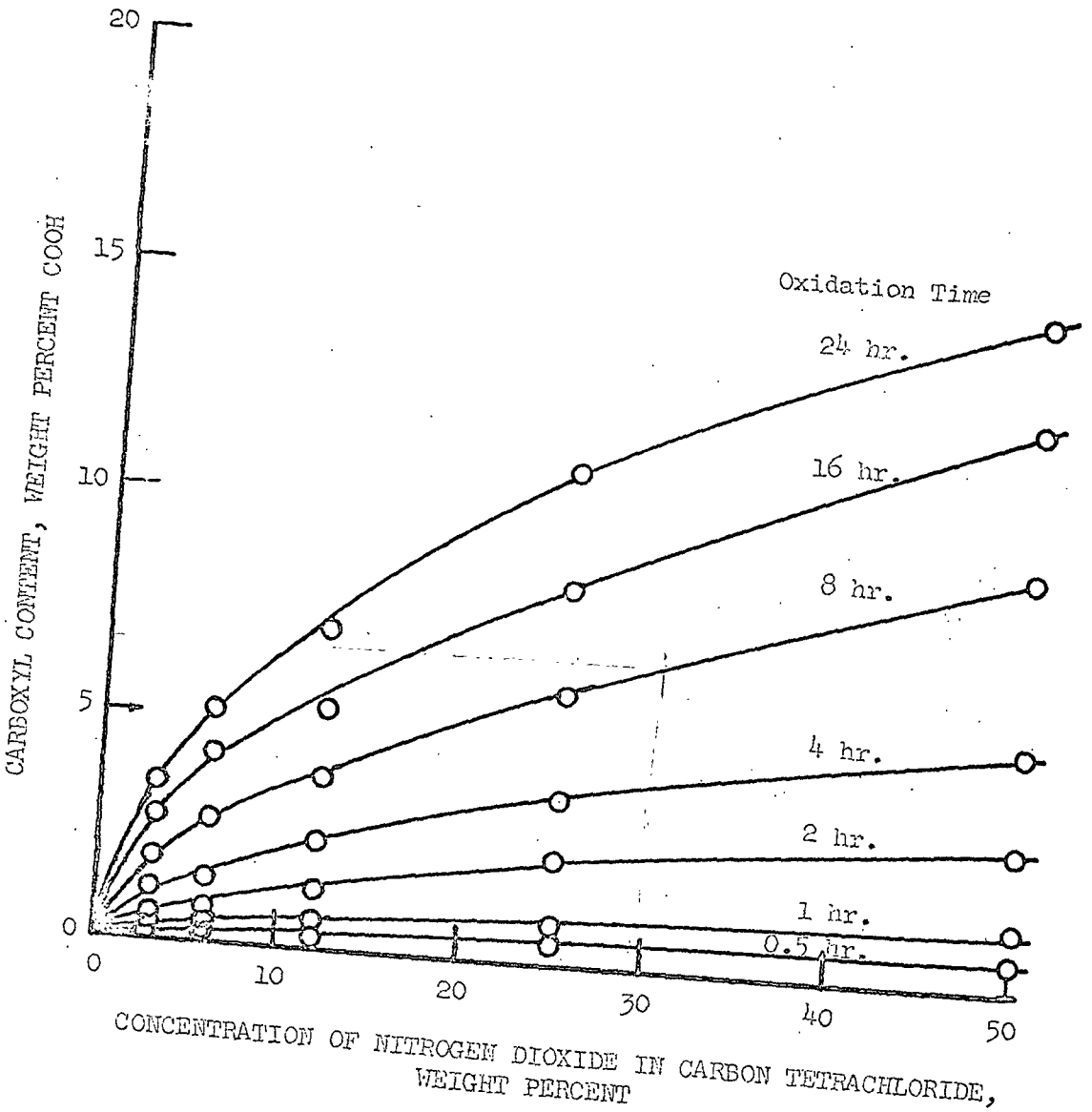

Figure 1

Effect of Nitrogen Dioxide Concentration and Time of Oxidation on Carboxyl Content of Celluronic Acids

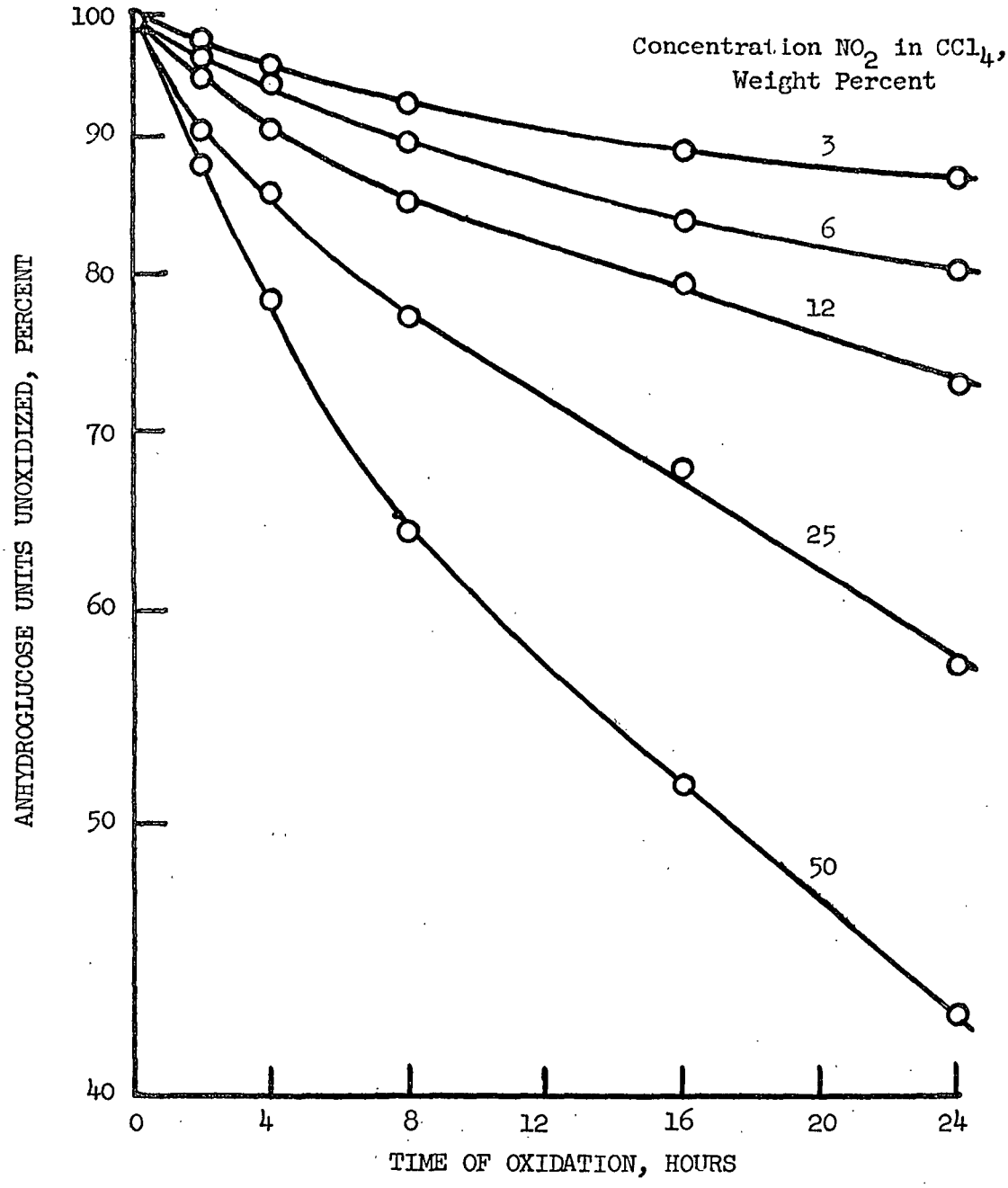

Figure 2

Effect of Concentration of Nitrogen Dioxide in Carbon Tetrachloride on Rate of Oxidation of Cotton Linters

previously reported data (16). However, it is interesting to note that rate of oxidation may be the sum of two first order reactions since a linear rate of oxidation is approached by all concentrations as the time of oxidation is increased. This would indicate that the second first order reaction prevailed on longer times of oxidation.

The data plotted in Figures 1 and 2 were obtained from oxidations in which a ratio of nitrogen dioxide-carbon tetrachloride solution to cellulose of 90:1 was used. Ratios of 135:1 and 50:1 were investigated, and found to produce the same degree of oxidation. The carboxyl contents of the celluronic acids produced using these ratios are tabulated in Table II.

From the data of these preliminary oxidation experiments, a concentration of 6% nitrogen dioxide in carbon tetrachloride and a ratio of oxidizing medium to cellulose of 50:1 were selected for use in subsequent experiments. These conditions were considered desirable since only low degrees of oxidation were obtained from oxidations extending over a reasonable period of time.

OXIDATION OF COTTON LINTERS WITH A SIX PERCENT SOLUTION OF NITROGEN DIOXIDE IN CARBON TETRACHLORIDE

REPRODUCIBILITY OF OXIDATION

The reproducibility of oxidation of cotton linters by nitrogen dioxide was first considered in order to establish (a) if the oxidation could be controlled, and (b) the exactness of technique and manipulation required. The criterion for reproducibility arbitrarily chosen was that

the carboxyl contents, as determined by the calcium acetate method, of the products from triplicate oxidations of cotton linters should not vary more than 0.10% carboxyl by weight. This limit was readily attainable, and the oxidations were considered reproducible. The carboxyl data for all celluronic acids produced may be found in Table III. The average carboxyl contents obtained at each oxidation period have been plotted in Figure 3.

FURTHER CHARACTERIZATION OF CELLURONIC ACIDS

In addition to carboxyl contents, the celluronic acids have been characterized by the following analyses, the results of which are included in Tables III to VI.

Nitrogen.

If nitrogen is introduced into the celluronic acids, the functional groups containing the nitrogen may be responsible for the properties noted. For each time of oxidation, one sample was analyzed for nitrogen by the Dumas method (27). The nitrogen contents found were low for all oxidation times. The nitrogen which has been introduced during oxidation can be present in either nitrate or nitrite form, as indicated by a positive diphenylamine test (28). Cotton linters gave a negative diphenylamine test before oxidation.

It has been concluded from the low nitrogen contents that the presence of combined nitrogen does not account for the anomalous properties of the celluronic acids.

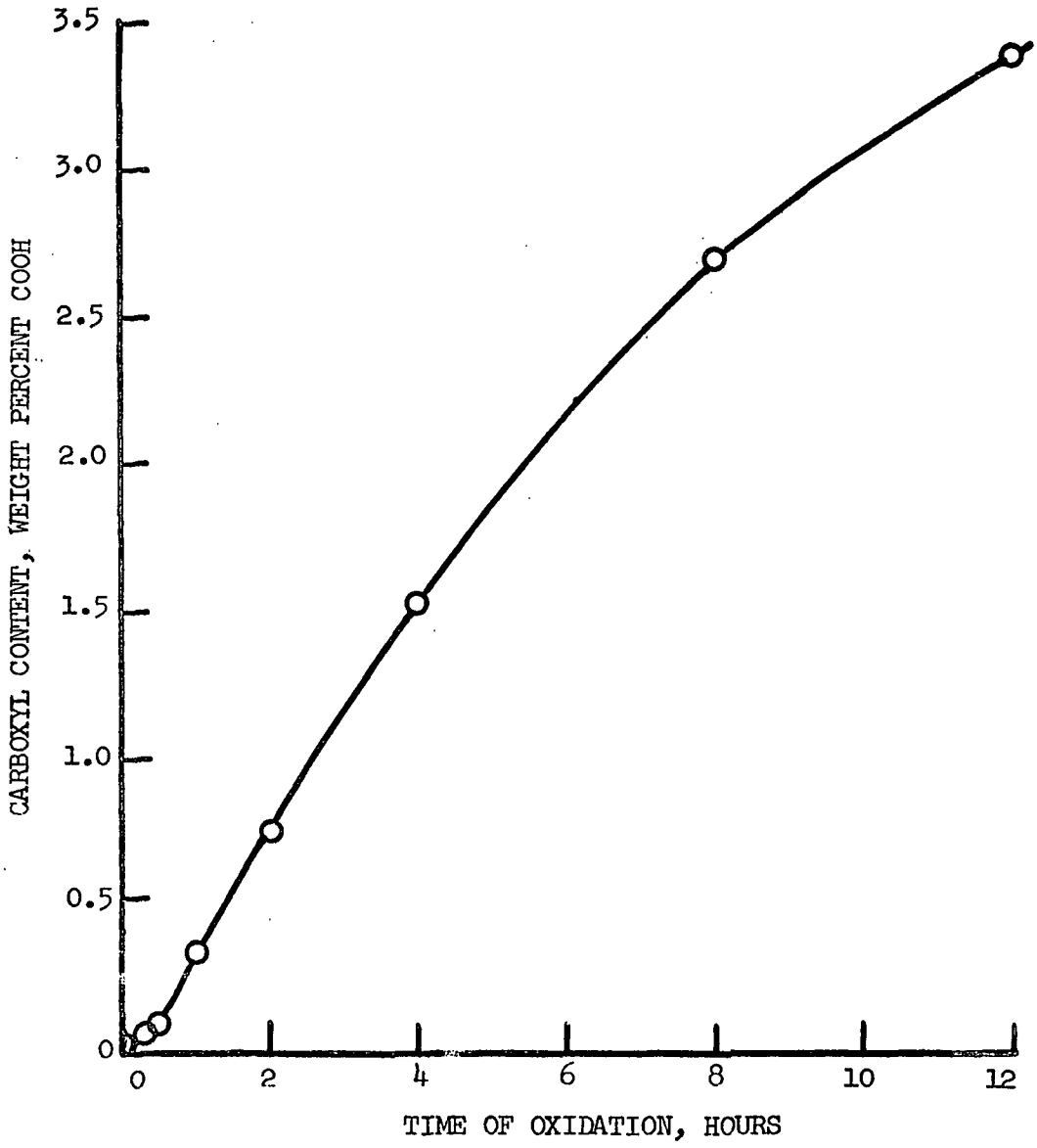

Figure 3

Carboxyl Content of Celluronic Acids
(Average values of triplicate oxidations at each time period)

Ash.

The salts of celluronic acids do not react with calcium acetate to form acetic acid in the carboxyl determination. The ash contents of the celluronic acids were determined as an indication of the number of carboxyl groups which were not in the free acid form. The ash contents of the celluronic acids were lower than the ash content of the original linters, with only one exception. These low ash values indicated that the carboxyl determination was essentially a measure of all carboxyl groups in the celluronic acids. The ash data are included in Table III.

A qualitative spectrographic analysis showed that the ash from the unoxidized linters contained calcium and magnesium. The ash from the celluronic acids contained copper, iron, and silver in addition to calcium and magnesium. Corrosion of the stirrers which were fabricated with silver solder accounts for the introduction of copper, iron, and silver into the celluronic acids.

Alkaline D.P.

The intrinsic viscosities of the celluronic acids were determined from cupriethylenediamine viscosity data. The intrinsic viscosity values were then converted to D.P. by multiplication by the factor 150 (29). An error is possibly introduced into the D.P. calculation by the use of the factor 150 which was determined for cellulose—not celluronic acids.

The D.P. data have been tabulated in Table III, and average values for each oxidation time plotted in Figure 4. The D.P. drops rapidly from

the original D.P. of 1600 of unoxidized linters to a leveling-off value of about 220. A statistical analysis indicated that the variation in D.P. between oxidation times of 2 to 12 hours is not significantly greater than the variation in D.P. within the same time of oxidation.

Alkali Solubility.

One of the outstanding properties of celluronic acids is their solubility in dilute alkali. Celluronic acids with 9 to 13% carboxyl and greater are completely soluble in 0.1 N sodium hydroxide at 20°C. (2,4). In order to determine the effect of dilute alkalies on celluronic acids of lower degrees of oxidation, the products of oxidation were treated with 0.100 N sodium hydroxide at 20°C. The soluble portion of each celluronic acid was calculated by difference from the weight of the residue and also from the wet oxidation of the material dissolved in solution. The results of both determinations on duplicate samples were in good agreement, and have been tabulated in Table IV. Better agreement between methods of computation would have been obtained if wet oxidations were not calculated on the basis of the molecular weight of anhydroglucose units. Since some oxidation of the anhydroglucose units had already occurred, the wet oxidation values as calculated are low. The average values calculated from the residue weights have been plotted in Figure 5. The alkali solubility of the celluronic acids is proportional to the time of nitrogen dioxide oxidation.

Carbonyl Content.

The carbonyl content of a series of celluronic acids was estimated

by the methylhydroxylamine method (24) and by copper number (30). The methylhydroxylamine method reportedly gives an absolute value of carbonyl, while a stoichiometric relation between copper number and carbonyl content does not exist.

Methylhydroxylamine hydrochloride was reacted with celluronic acids in the free acid form and in the calcium salt form. After 24 hours at 20°C. the celluronic acids were filtered off, and an aliquot of the filtrate titrated to measure the liberated hydrochloric acid. The celluronic acids were then washed with water and subjected to Kjeldahl nitrogen analysis (31). The data have been plotted in Figure 6 and tabulated in Table V. Curve 1 of Figure 6 represents the total acid liberated during reaction of methylhydroxylamine hydrochloride with free celluronic acids according to the following reactions (24).

$$R_2CO + HCl \cdot H_2NOCH_3 = R_2CNOCH_3 + H_2O + HCl \qquad (1)$$

$$RCOOH + HCl\text{-}H_2NOCH_3 = RCOOH \cdot H_2NOCH_3 + HCl \qquad (2)$$

Assuming that each carboxyl group liberated a molecule of acid, Curve 1 corresponds to the total carbonyl plus carboxyl. Curve 2 is obtained by subtracting the number of carboxyl groups determined by the calcium acetate method from the total (Curve 1); it should represent the carbonyl groups only. The data from nitrogen analysis of the washed celluronic acids gave Curve 3.

When reacted with the calcium salt of celluronic acids, methylhydroxylamine is reported to measure only carbonyl groups (24). It is more likely that, at the pH 4.6 of the reaction, the calcium salts are at

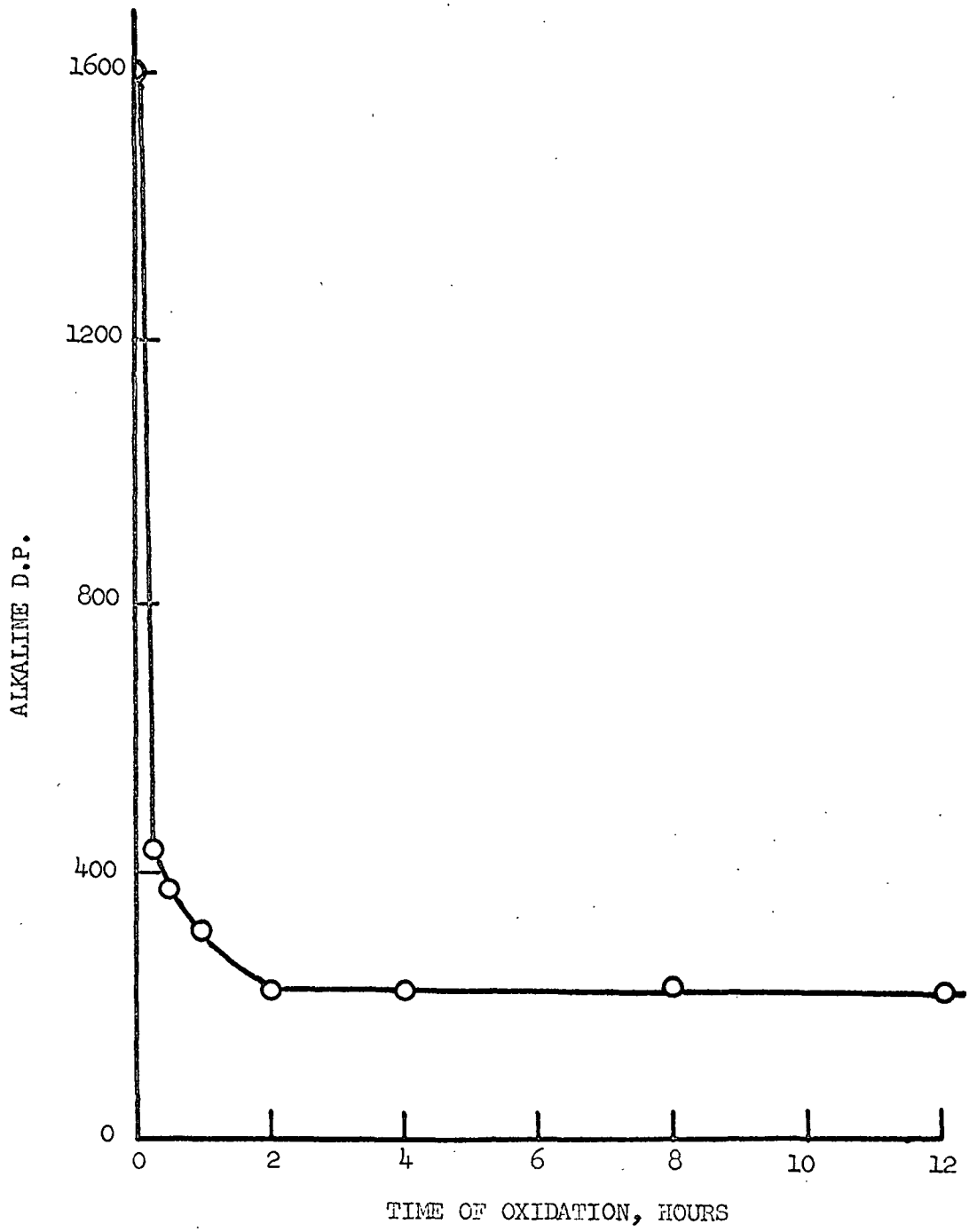

Figure 4

Alkaline D.P. of Celluronic Acids
(Average values of triplicate oxidations at each time period)

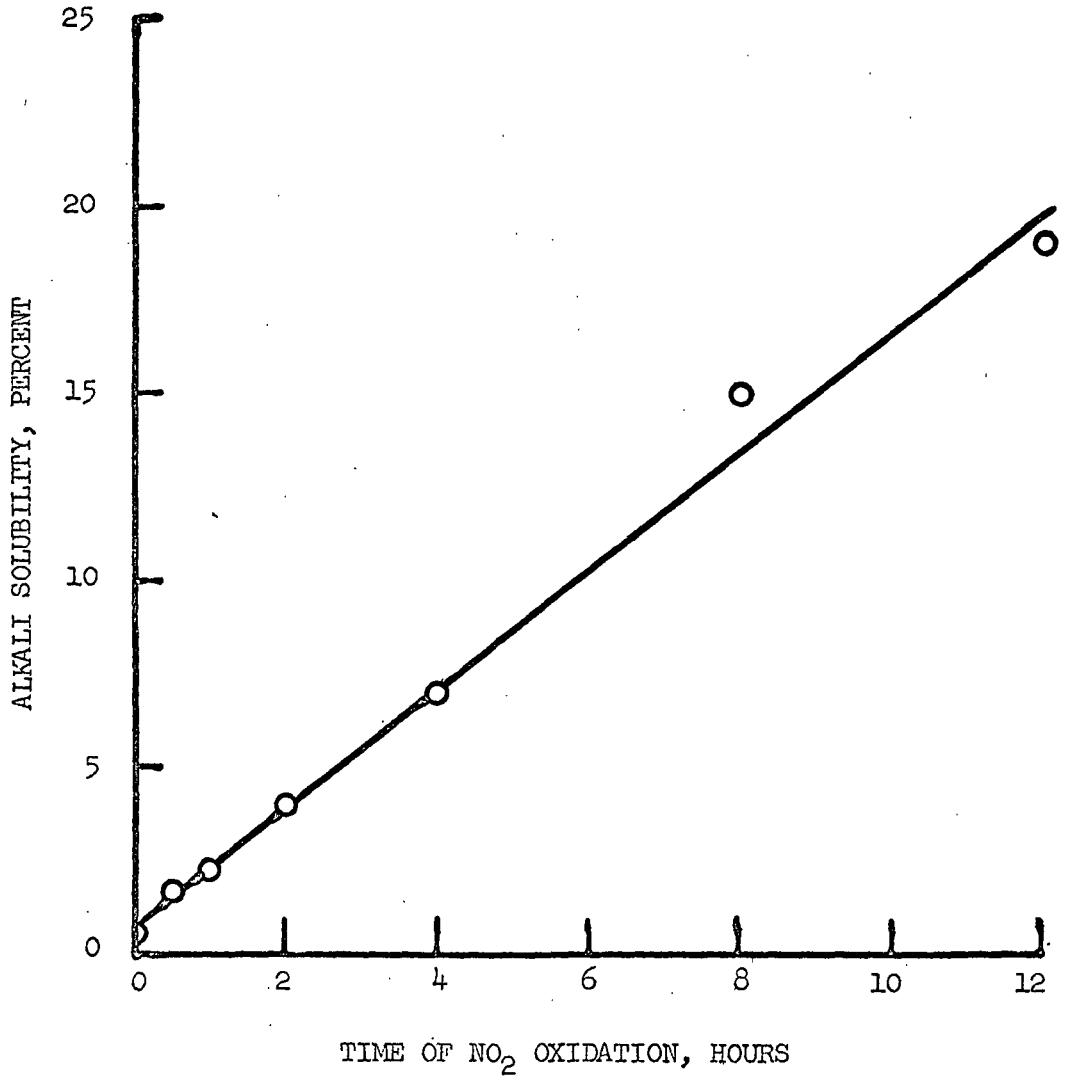

Figure 5

Solubility of Celluronic Acids in 0.100 N Sodium Hydroxide

least partly dissociated and that some methylhydroxylamine reacts according to Equation 2 above. Curve 4 represents the titration of liberated acid from the calcium salts of the celluronic acids and is a measure of the carbonyl groups plus a portion of the carboxyl groups. Nitrogen values on the washed residues yielded Curve 5.

Curves 2, 3 and 5 indicate that the same order of magnitude of substitution is obtained by the three analyses. Since Curve 2 represents carbonyl groups, it is likely that the nitrogen analyses represented by Curves 3 and 5 also correspond to carbonyl. For this to be the case, washing the celluronic acids with water would have to remove all methylhydroxylamine associated with carboxyl groups. Only nitrogen introduced by reaction of methylhydroxylamine with carbonyl groups would then be determined by Kjeldahl analysis.

As a check that washing removes methylhydroxylamine associated with carboxyl groups, duplicate samples of 8 and 12-hour oxidation products were subjected to repeated reaction with methylhydroxylamine alternated with thorough washing with water. It was expected that during the first reaction period all carbonyl groups and the reacting portion of the carboxyl groups liberated hydrochloric acid for titration. Subsequent reactions should only liberate acid in a quantity representing reaction with carboxyl groups. Both the second and third treatments with methylhydroxylamine hydrochloride liberated the same amount of acid, but it corresponded to only about half of the carboxyl groups as estimated by the calcium acetate method. The average data for each celluronic acid are in Table VI. This experiment raises a doubt that

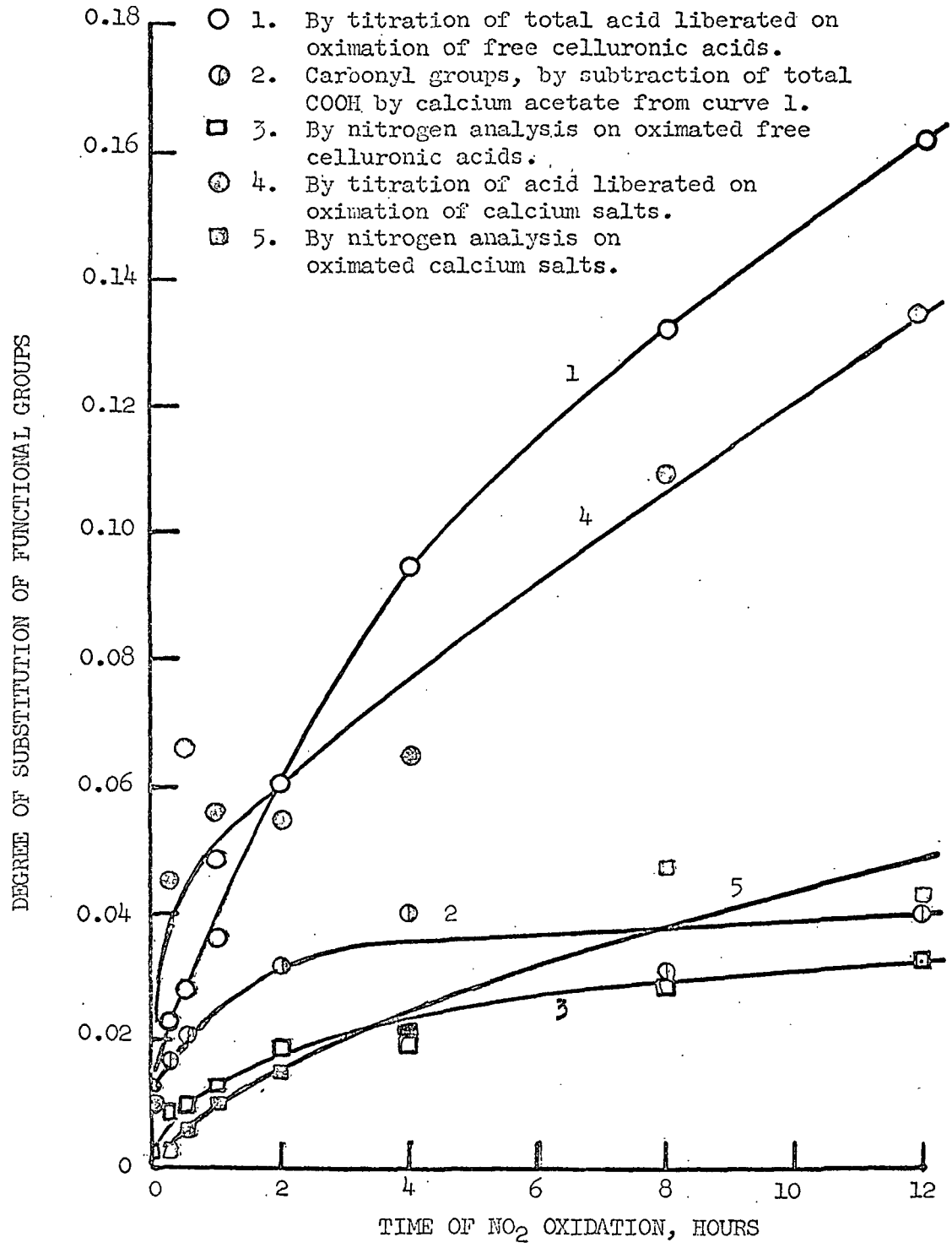

Figure 6

Methylhydroxylamine Hydrochloride Reaction with Celluronic Acids

the reactions with methylhydroxylamine hydrochloride occur as postulated in Equations 1 and 2 above or that washing consistently removes methylhydroxylamine held as a salt. This result was not anticipated.

The copper numbers of the celluronic acids offer an empirical estimation of the carbonyl content. In an effort to see if copper number can be correlated with carbonyl content, the copper numbers of the celluronic acids which were reacted with methylhydroxylamine have been plotted in Figure 7 against the carbonyl contents determined from nitrogen analyses (Curve 3 of Figure 6). A correlation does exist, and can be used as an indication of the degree of substitution of carbonyl groups for other celluronic acids whose copper number has been determined.

STORAGE OF CELLURONIC ACIDS

The celluronic acids prepared during the study of the reproducibility of nitrogen dioxide oxidation were stored in the free acid form in closed bottles for later modification and characterization studies. Instability of celluronic acids has been suspected (4). Accordingly, estimations of carboxyl content and alkaline D.P. were made before and after 10 to 39 weeks. The carboxyl data are listed in Table VII and the alkaline D.P. data in Table VIII.

The changes which occurred in storage were not a function of the level of oxidation. The decreases in carboxyl and D.P. were of the same order of magnitude regardless of the original values. No suitable explanation for these changes has been developed in the present work;

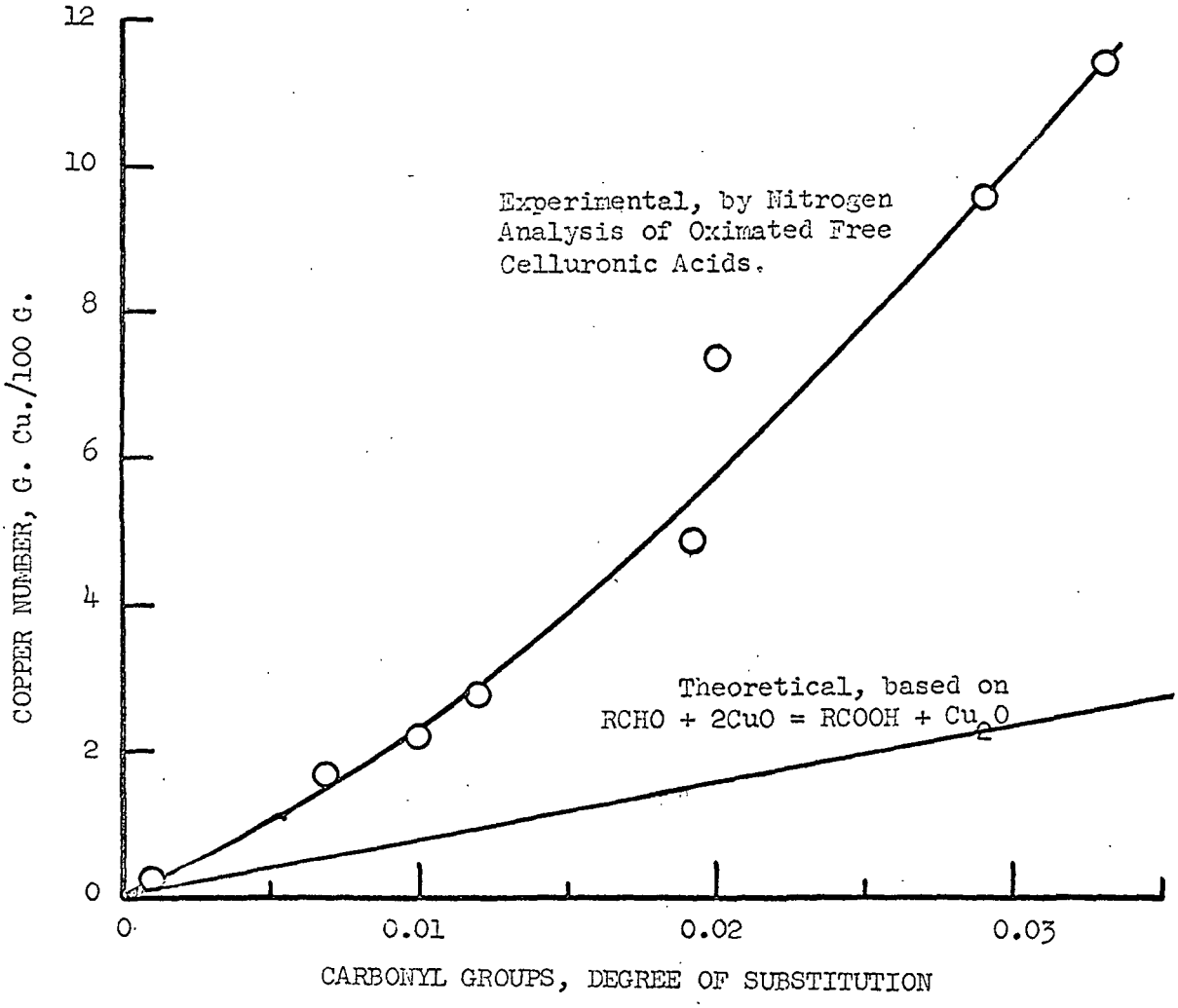

Figure 7

Correlation between Copper Number and Carbonyl Content

however, similar changes have previously been attributed to autohydrolysis (32).

The changing carboxyl content and alkaline D.P. indicate that all modification and characterization analyses should be conducted either immediately after preparation of the celluronic acids, or with repeated reference to the celluronic acids in order to account for storage changes. Since it was not possible to completely characterize the celluronic acids immediately after preparation, the data reported henceforth have been suitably paired to reflect changes from the celluronic acids as they occurred at the time of modification or analysis.

MODIFICATION OF CELLURONIC ACIDS

A method of D.P. determination which is independent of the effect of alkali-sensitive groups is essential for demonstrating whether or not degradation has occurred during the oxidation of cellulose with nitrogen dioxide. The classical method for determining the D.P. of cellulose in nonalkaline solvents has been the measurement of the viscosities of the nitrates in acetone or ethyl lactate.

The nitrates of celluloses oxidized with chromic acid (13), hypobromite, and hypochlorite (20) were soluble at all levels of oxidation. Moreover, the nitrates of pectin, a polygalacturonic acid in which a portion of the carboxyl groups appear as methyl esters, have been used successfully for D.P. determination (34,35). Hence, it is not unreasonable to extend the nitrate viscosity method to celluronic acids.

The nitrates of periodate-oxidized cellulose (26,33) and nitrogen dioxide-oxidized cellulose (4), however, are only partially soluble in acetone, except at extremely low levels of oxidation. When the aldehyde groups in periodate-oxidized celluloses were oxidized to carboxyl groups with chlorous acid before nitration, the nitrates became soluble (26).

These facts suggest the following experiments to arrive at soluble nitrates which will be suitable for D.P. determinations of celluronic acids in neutral organic solvents:

1. If carboxyl groups are the cause of insolubility, the esterification of these groups with diazomethane should eliminate their influence. An esterified celluronic acid would be analogous to pectin, except for the stereochemical difference between alpha-1,4-polyanhydrogalacturonic acid and beta-1,4-polyanhydroglucuronic acid.

2. If aldehyde groups introduced into the celluronic acids by side reactions are the cause of insolubility, the chlorous acid oxidation of these aldehyde groups to carboxyl groups should lead to soluble nitrates.

3. If carbonyl groups, both ketone and aldehyde groups, are important in causing insolubility of nitrates, the reduction of these groups with sodium borohydride should remove their influence.

Accordingly, celluronic acids have been variously treated prior to nitration, i.e., they have been either esterified with diazomethane, oxidized by chlorous acid, or reduced with sodium borohydride. In

addition to the solubility of the nitrates, the alkaline D.P., alkali solubility, carboxyl content, and copper number of the oxidized and reduced celluronic acids have been measured to characterize the products.

DIAZOMETHANE ESTERIFICATION OF CELLURONIC ACIDS

A 12-hour nitrogen dioxide oxidation product was treated with diazomethane in anhydrous ether for 1 to 8 hours. The esterification of the carboxyl groups was not complete, although the free acid content was reduced from 3.45 to 0.58%. The analyses are tabulated in Table IX.

Diazomethane treatment of periodate- oxidized celluloses has been reported to decrease alkali sensitivity (37). When a celluronic acid was methylated 48 hours in the present experiments a change in alkaline D.P. did not occur. Furthermore, this celluronic acid was not completely soluble in cupriethylenediamine at concentrations above 0.25 grams per 100 ml. This is similar to insolubilities which have been reported for methylated celluronic acids in cuprammonium (36).

The methylated products, when nitrated, were not completely soluble in acetone or ethyl lactate. From this experiment it was concluded that if carboxyl groups cause insolubility of the nitrates, only a small number are required. However, it has been reported that pectin, with as low as 9% of the carboxyl groups esterified (91% in free acid form), was soluble in acetone when nitrated (35). Thus, it appears that esterification is not a requisite for the solubility of celluronic acid nitrates.

CHLOROUS ACID OXIDATION OF CELLURONIC ACIDS

Chlorous acid oxidizes aldehyde groups to carboxyl groups (25,26). After chlorous acid oxidation, celluronic acids had an increased carboxyl content and a decreased copper number (4). However, all carbonyl groups present in the celluronic acids were not reactive aldehydes, for an appreciable copper number was retained after chlorous acid oxidation. This copper number could have been due to ketone groups which gave rise to reducing groups after chain degradation in the hot alkaline copper reagent. Similar results have been found in the present experiments. It has further been found that chlorous acid oxidation stabilized the celluronic acids to cupriethylenediamine to some extent, because alkaline D.P. was increased. The property changes which occurred during chlorous acid oxidation of celluronic acids in the present experiments have been tabulated in Table X. The data from which these changes have been calculated are in Table XI. The implications of the data will be discussed later.

The removal of aldehyde groups from periodate-oxidized celluloses by chlorous acid oxidation resulted in materials whose nitrates were soluble in acetone (26). However, in the case of celluronic acids, it has now been found that chlorous acid oxidation does not lead to nitrates which are completely soluble in acetone and ethyl lactate. The ketone groups as well as the aldehyde groups which may be introduced during nitrogen dioxide oxidation apparently have a prominent role in preventing the complete solubilization of the nitrates.

SODIUM BOROHYDRIDE REDUCTION OF CELLURONIC ACIDS

The properties of celluronic acids reduced with sodium borohydride have not previously been investigated. However, investigations on periodate-oxidized celluloses (38) and hydrocelluloses (39) indicated that stabilization to alkali could be obtained by sodium borohydride reduction of carbonyl groups to hydroxyl groups. Since the greatest stabilization of the periodate-oxidized celluloses and hydrocelluloses was obtained in an unbuffered aqueous solution of sodium borohydride at 20°C., these conditions were adopted for the reduction of celluronic acids. A 0.05 molar solution of sodium borohydride was found to be most efficient for reducing celluronic acids. With a 0.05 molar solution the greatest increase in the alkaline D.P. of a celluronic acid was obtained; therefore, this concentration was used for subsequent reduction experiments. The D.P. increase obtained with various concentrations of sodium borohydride are tabulated in Table XII.

Since sodium borohydride reduces carbonyl groups without affecting free carboxyl groups (40), this reagent should not affect carboxyl groups in celluronic acids*. In the present experiments, the majority of the carboxyl groups remained after reduction. Furthermore, the copper number of the celluronic acids was decreased while the alkaline D.P. was increased--indicating that reduction stabilized the celluronic acids to alkali. The changes which occurred on reduction are tabulated in Table X and were calculated from the data of Table XI. A loss of yield of up to 8% occurred during reduction.

* Any lactones present may be reduced to primary hydroxyl groups by sodium borohydride.

The nitrates derived from reduced celluronic acids were found to be completely soluble in acetone and ethyl lactate. The pH of the unbuffered sodium borohydride solution was slightly alkaline; therefore, either the removal of alkali-soluble material or the reduction of carbonyl groups could have been responsible for the changes which result in soluble nitrates.

To demonstrate which effect, reduction or removal of alkali-soluble material, predominated, a special experiment was conducted. In this experiment, the pH of an aqueous suspension of a celluronic acid was maintained at 9.7 through frequent additions of 0.1 N sodium hydroxide. This pH paralleled the pH during reduction of samples of the same celluronic acid. The results of this experiment are tabulated in Table XIII.

This experiment indicated that, although a greater loss of yield occurred on reduction, greater stabilization also occurred. In the samples whose pH was maintained with sodium hydroxide, appreciable alkaline degradation did not occur. The slight increase in alkaline D.P. indicates that only short chain molecules were removed. Reduction, on the other hand, resulted in an appreciable increase in alkaline D.P. and decrease in copper number. Therefore, it has been concluded that the alkaline pH of reduction was not responsible for the modifications of the celluronic acids which resulted in soluble nitrates.

PROPERTIES OF CELLURONIC ACIDS AND MODIFIED CELLURONIC ACIDS

The modified celluronic acids have been characterized through

alkaline D.P., copper number, and alkali solubility. The celluronic acids have been similarly characterized at the same time to account for aging effects.

In Figure 8, the alkaline D.P. of the celluronic acids (Curve 1), chlorous acid-oxidized celluronic acids (Curve 2), and reduced celluronic acids (Curve 3) have been plotted as a function of the time of nitrogen dioxide oxidation. The alkaline D.P. of the celluronic acids decreased rapidly during the first 2 hours of oxidation to a leveling-off value. The alkaline D.P. of celluronic acids (produced by less than 8 hours nitrogen dioxide oxidation) modified by chlorous acid oxidation is greater than the D.P. of the unmodified celluronic acids; and the D.P. of the celluronic acids modified by reduction is still greater.

The D.P. of the alkali-extracted (Figure 8a) celluronic acids (Curve 1) and modified celluronic acids (Curves 2 and 3) were lower than the D.P. of the unextracted acids. The reduced celluronic acids retained the greatest resistance to degradation during alkaline extraction.

If the alkali-soluble material removed during extraction with 0.100 N sodium hydroxide were of low D.P. only, the D.P. of the extracted residues would have shown a slight increase. Since a decrease in D.P. occurred, longer chains must have been cleaved. This indicates that a greater amount of degradation occurred during extraction with 0.100 N sodium hydroxide than in cupriethylenediamine or alkaline solutions of pH 9.7. Although the conditions of alkaline extraction were mild, some air oxidation may have occurred.

The modifications of the celluronic acids resulted in the removal of carbonyl groups, as noted by decreased copper numbers. The effect of chlorous acid oxidation and of reduction on the copper number of the celluronic acids is illustrated in Figure 9 which was prepared from the data of Table XI. All carbonyl groups were not reduced by sodium borohydride since reduced celluronic acids retained small copper numbers.

The alkali solubilities of the celluronic acids (Curve 1) and modified celluronic acids are illustrated in Figure 10. Chlorous acid oxidation had little effect on alkali solubility (Curve 2). Reduction, on the other hand, decreased alkali solubility (Curve 3) markedly even when corrected for losses which occurred during reduction (Curve 4). Since the yield during chlorous acid oxidation was always 99.0% or better, correction for the loss is small and has been omitted from Figure 10. The data from which this figure was prepared are in Tables XIV to XVI.

The relationship between changes in copper number, alkaline D.P., and alkali solubility which occurred on modification of the celluronic acids have been plotted in Figure 11 from the data of Table X and Tables XIV to XVI. In this figure, delta alkali solubility represents the difference between (1) the alkali solubility of unmodified celluronic acids and (2) the total losses during modification and alkali extraction of the modified celluronic acids. This is the same as the difference between Curves 1 and 2 or Curves 2 and 4 of Figure 10. In Figure 11, the delta alkaline D.P. values are the same as differences between Curves 1 and 2 or Curves 1 and 3 of Figure 8, while delta copper number values are the same as differences between Curves 1 and 2 or Curves 1 and 3 of Figure 9.

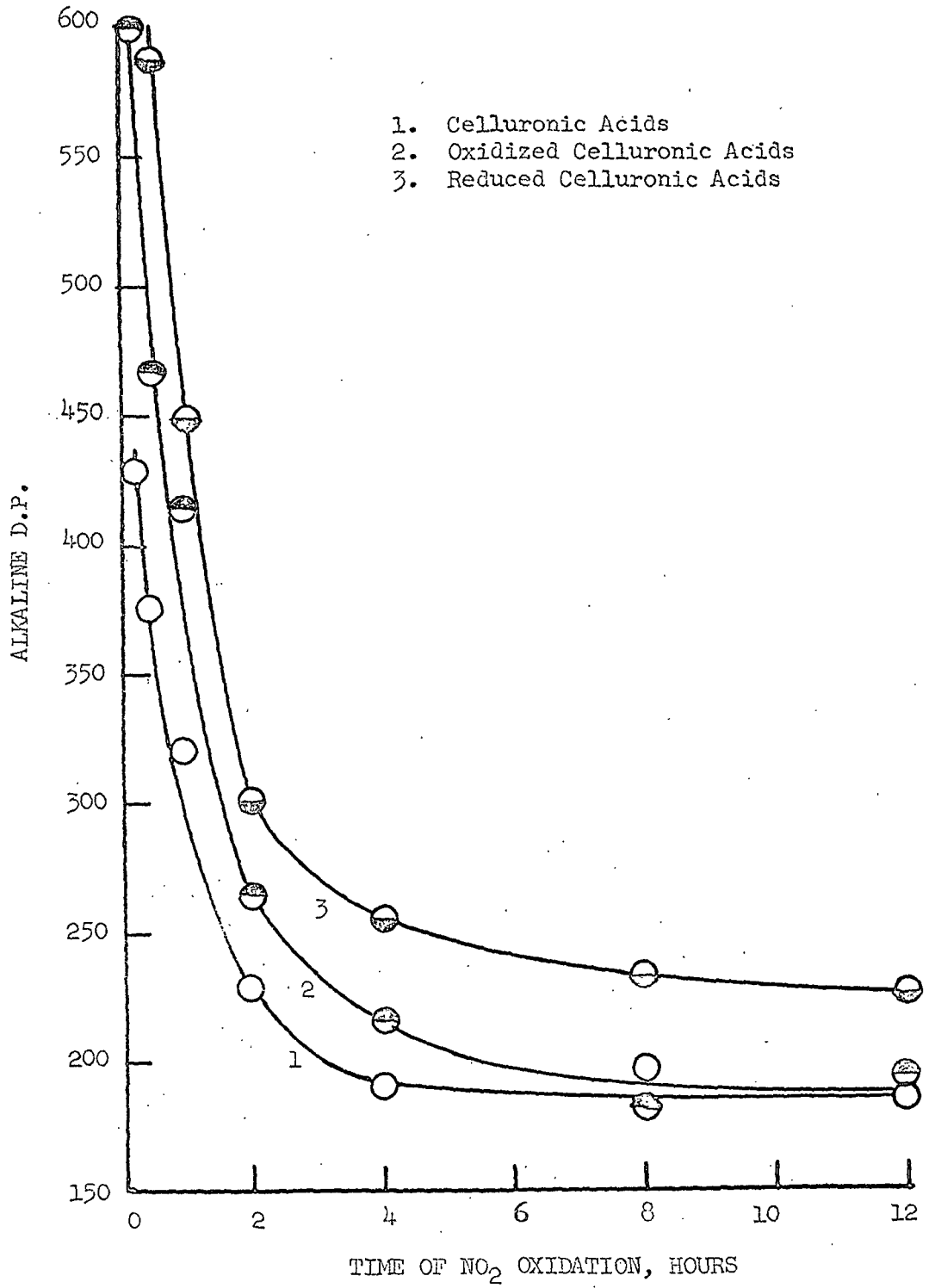

Figure 8

Alkaline D.P.

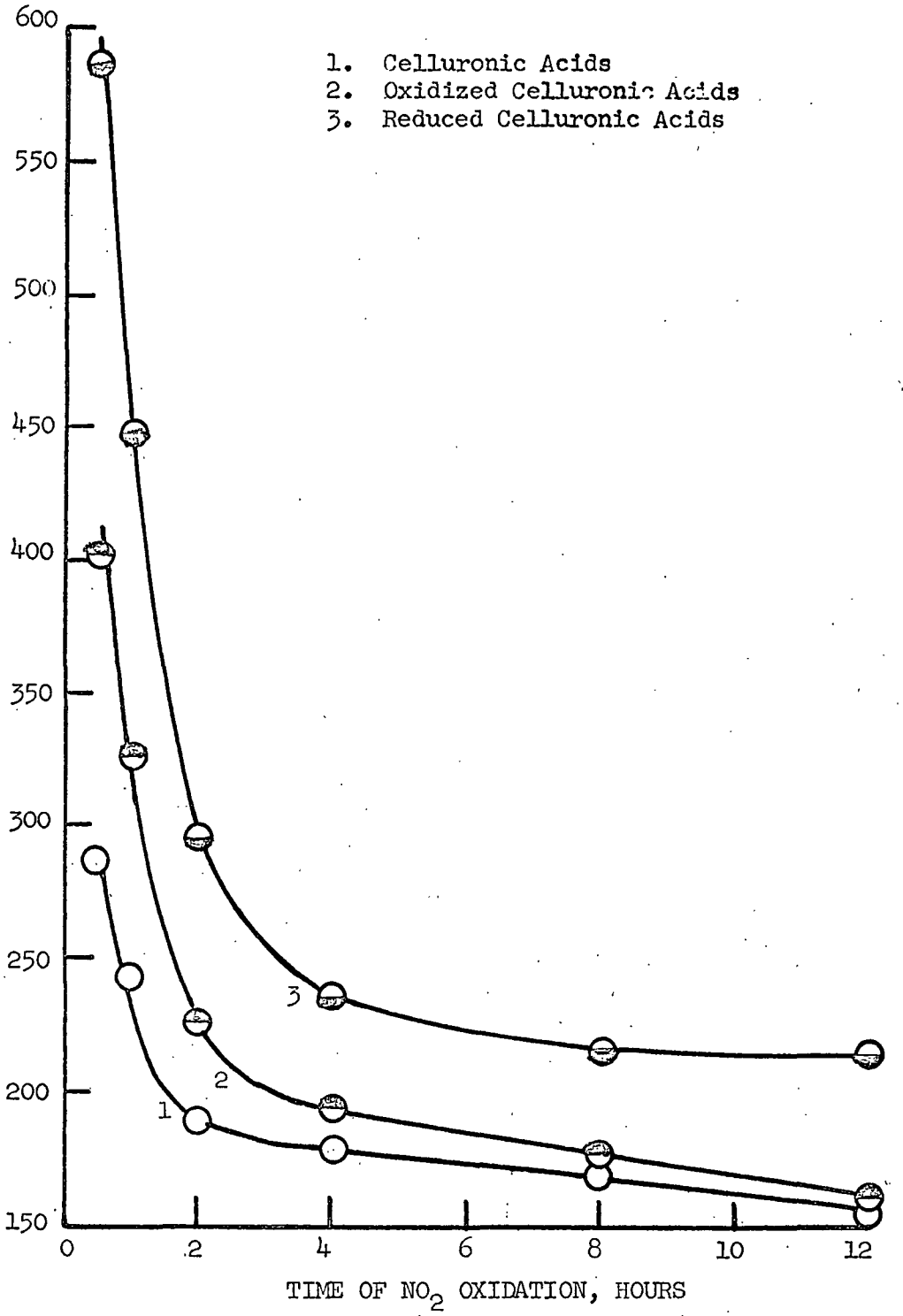

Figure 8a

Alkaline D.P. after Alkali Extraction

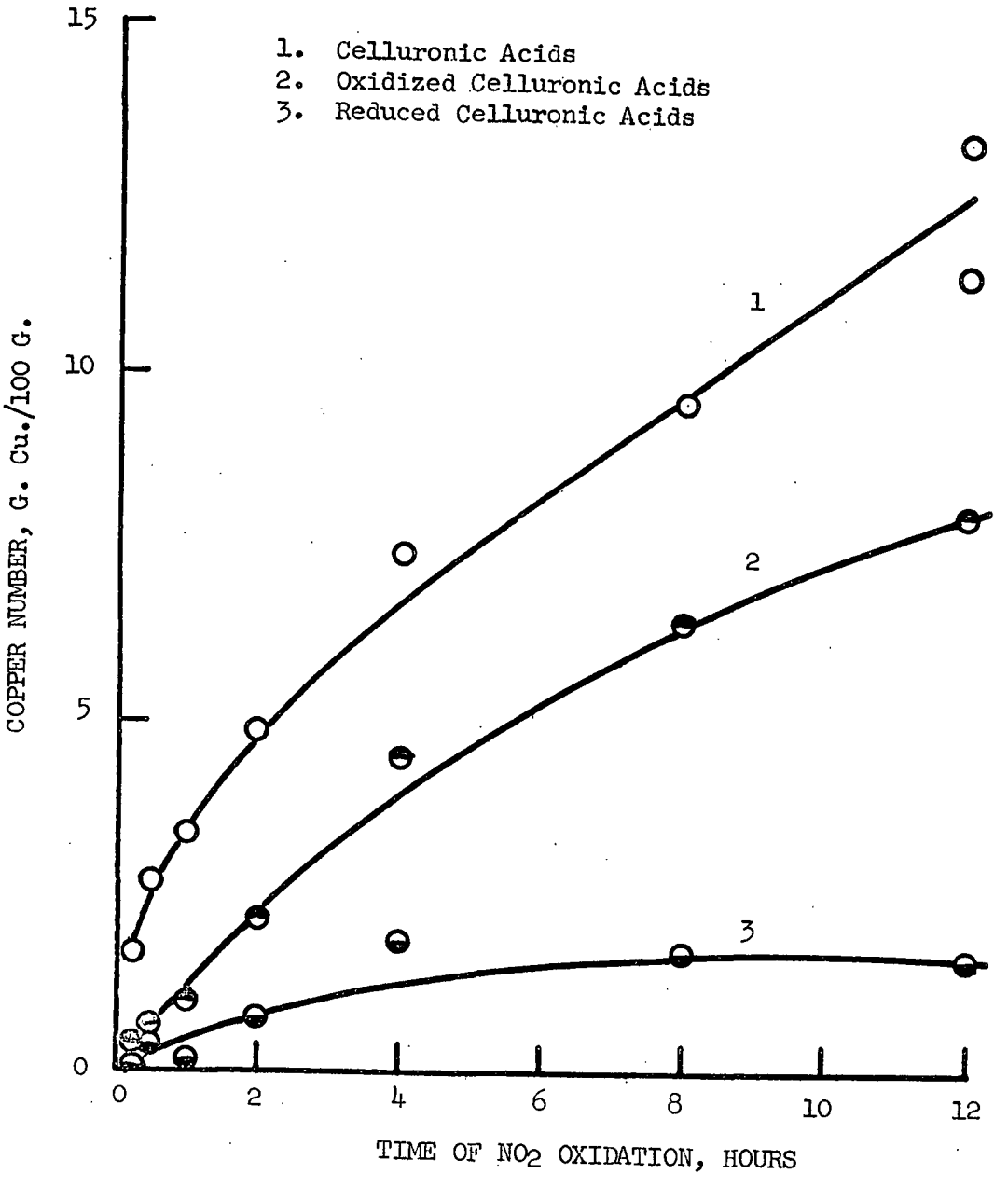

Figure 9

Copper Number

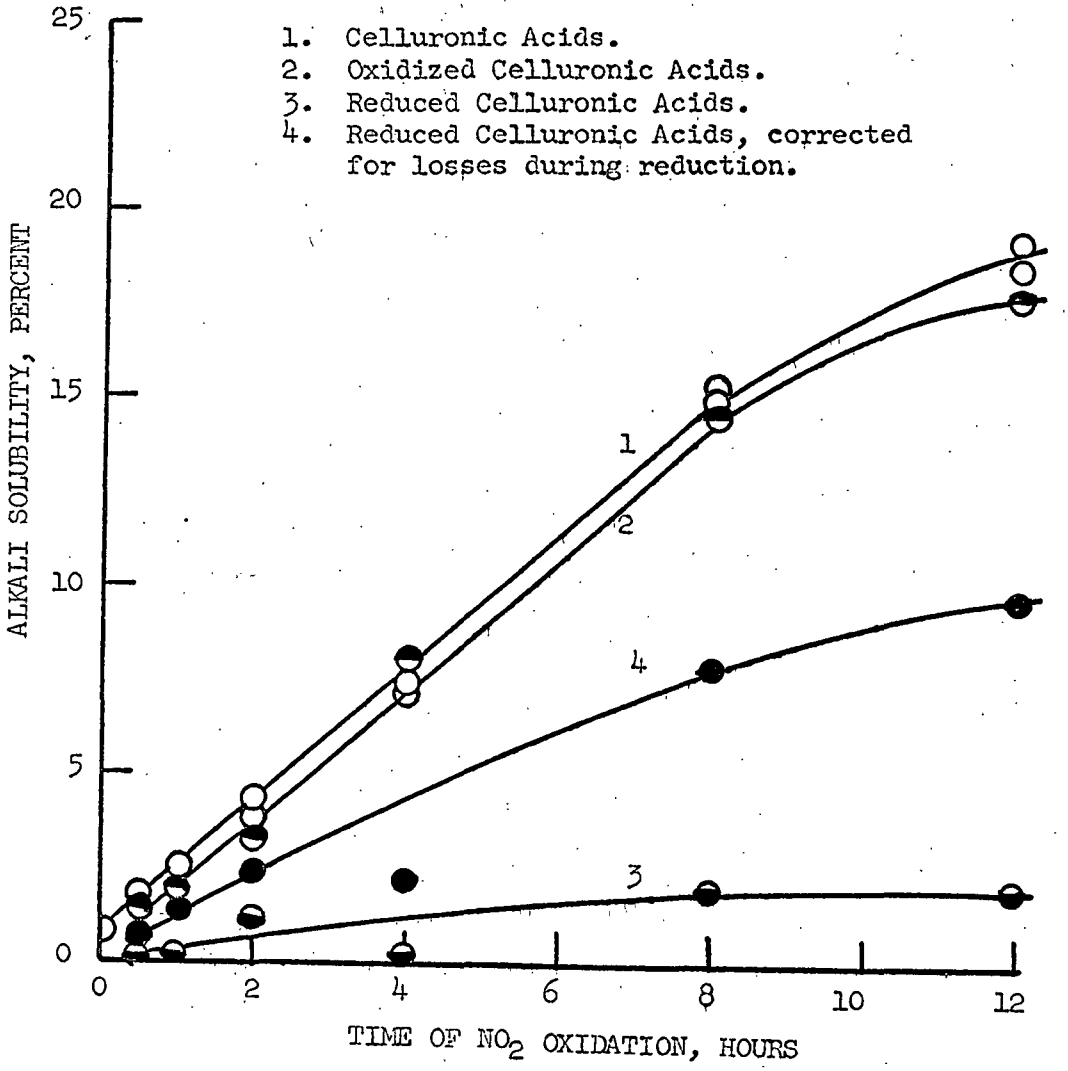

Figure 10

Solubility in 0.100 N Sodium Hydroxide

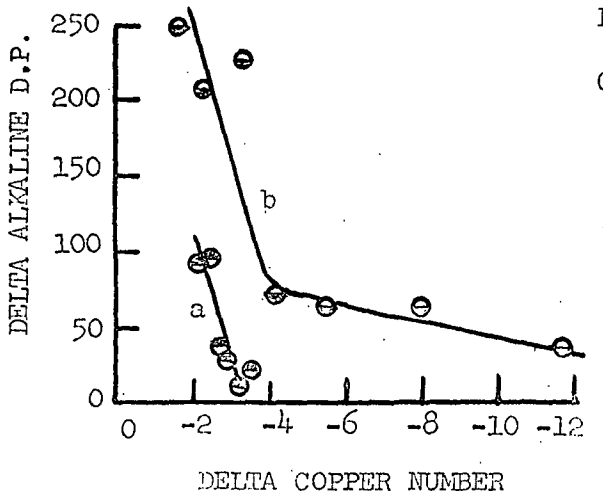

Figure 11a

Changes in Alkaline D.P. and Copper Number on
a. Oxidation with $HClO_2$
b. Reduction with $NaBH_4$

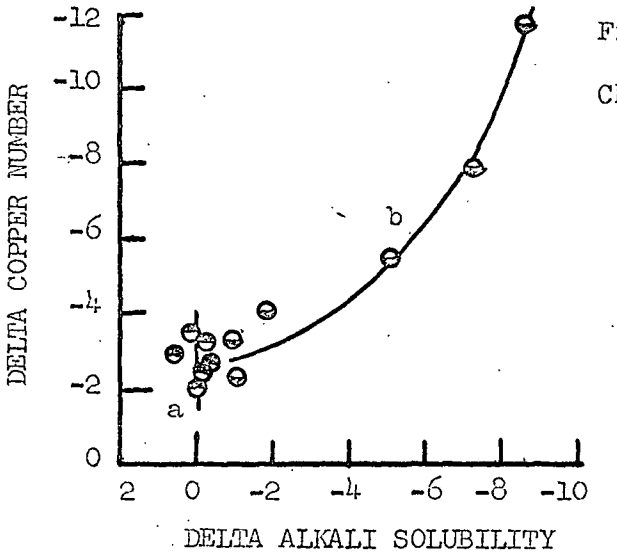

Figure 11b

Changes in Copper Number and Alkali Solubility on
a. Oxidation with $HClO_2$
b. Reduction with $NaBH_4$

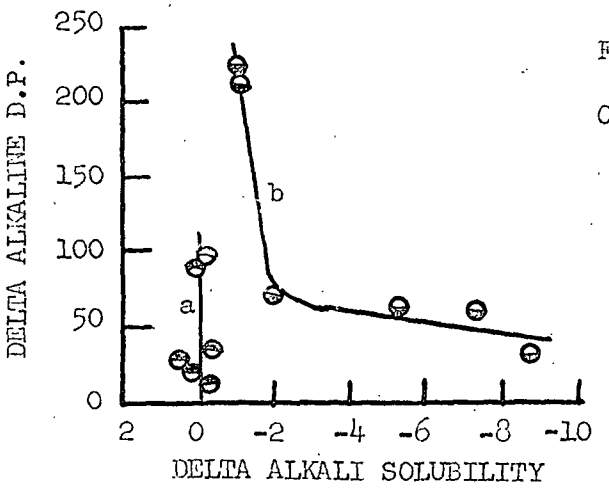

Figure 11c

Changes in Alkaline D.P. and Alkali Solubility on
a. Oxidation with $HClO_2$
b. Reduction with $NaBH_4$

Figure 11

Changes in Properties of Celluronic Acids

Aldehyde groups which were oxidized by chlorous acid apparently did not affect the alkali solubility of celluronic acids, although their oxidation did contribute to an increase in alkaline D.P. Some of these aldehyde groups may have been terminal, but others must have been located on chains whose fragments after scission were not soluble in 0.100 N sodium hydroxide. This relation is apparent from Curve a of Figure 11c, which shows that the increasing alkaline D.P. was not accompanied by a change in alkali solubility.

The alkaline extraction of celluronic acids revealed that cupriethylenediamine, 0.100 N sodium hydroxide at 20°C., and hot copper reagent had increasing effect on carbonyl degradation. Alkaline extraction reduced cupriethylenediamine D.P., indicating that chain scissions occurred during extraction beyond those which may have occurred in cupriethylenediamine. The copper numbers after extraction (Figure 12-Curve B) indicate that additional reducing groups remained for reaction with this reagent.

Figures 12 and 13 indicate the distribution of carbonyl groups and carboxyl groups of celluronic acids between the alkali-extracted residues and the alkali-soluble portions. The copper number and carboxyl content for the celluronic acids and alkali-extracted residues were determined experimentally. The copper number and carboxyl content of the alkali-soluble fractions were calculated by difference from the experimental values and the alkali solubility of the celluronic acids. These differences are small, and calculations based on them magnify experimental error. This accounts for the scatter encountered in the copper number

and carboxyl content of the alkali-soluble portion of the celluronic acids. The data from which Figures 12 and 13 were prepared have been tabulated in Table XVII.

Figures 12 and 13 demonstrate that the portions of the celluronic acids which are soluble in dilute alkali contain a higher percentage of carbonyl and carboxyl groups than the residues. Therefore, the most highly oxidized portions of the celluronic acids are those portions which are most readily extracted by alkali.

During the alkaline extraction of the celluronic acids a distinct yellow coloration was observed on addition of the sodium hydroxide. The colored material formed was soluble and was extracted. Alkaline extractions of celluronic acids modified by chlorous acid oxidation or sodium borohydride reduction were not accompanied by this yellow coloration.

SUMMARY: MODIFICATION OF CELLURONIC ACIDS

The alkaline D.P. of celluronic acids falls from the D.P. of linters during the first 2 hours of oxidation to leveling-off value of about 200. During this period of rapid decrease in D.P., less than 1% carboxyl is introduced. After the celluronic acids have been modified by chlorous acid oxidation or by sodium borohydride reduction, the alkaline D.P. is greater than the D.P. of unmodified celluronic acids. This greater D.P. is apparently due to the removal of carbonyl groups and the potential chain scission which could occur in alkaline cupriethylenediamine.

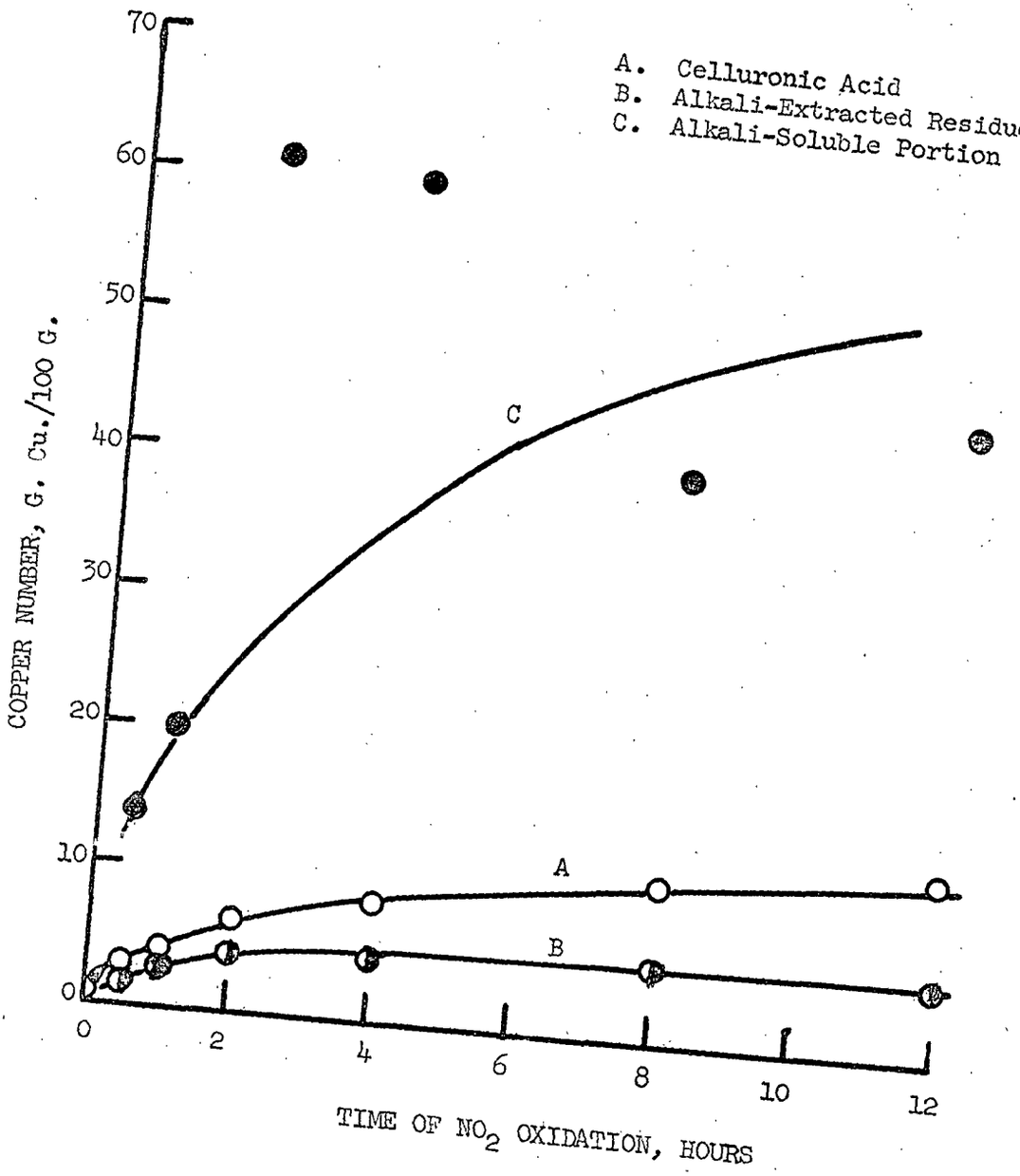

Figure 12

Distribution of Copper Number on Alkali Extraction of Celluronic Acids

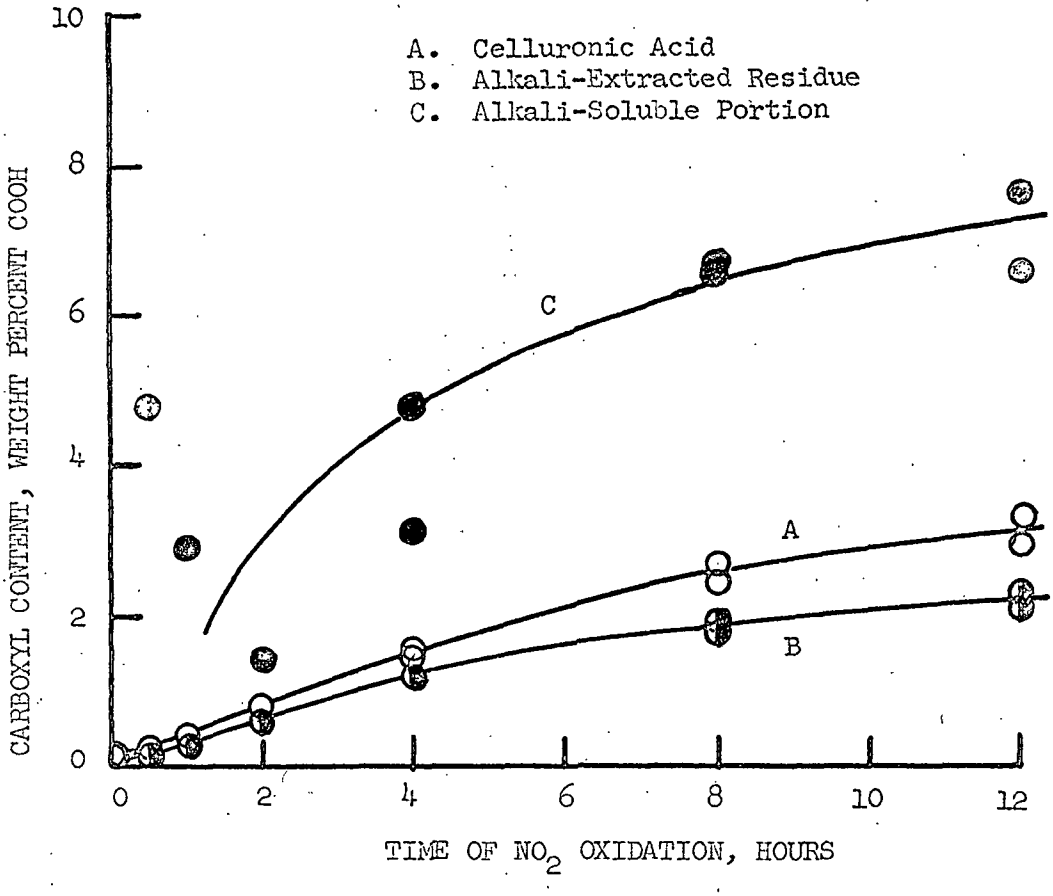

Figure 13

Distribution of Carboxyl Groups on Alkali Extraction of Celluronic Acids

Chlorous acid oxidations of the celluronic acids were carried out with a yield loss of 1% or less. In addition to maintaining a slightly higher alkaline D.P., chlorous acid oxidation also increased the carboxyl content and decreased the copper number of the celluronic acids. All of these changes indicate that aldehyde groups present in the celluronic acids were oxidized to carboxyl groups. The alkali solubility of the celluronic acids was virtually unaffected by chlorous acid oxidation.

Reduction of celluronic acids was accompanied by a yield loss up to 8% for a celluronic acid prepared by oxidation with nitrogen dioxide for 12 hours. Free carboxyl groups are not reduced by sodium borohydride and are retained in the modified celluronic acids. The copper number and alkali solubility were decreased and the alkaline D.P. maintained higher by reduction. A less extensive retention of D.P. occurred with more highly oxidized celluronic acids.

Alkaline chain scission at carbonyl groups in the viscosity solvent offers only a partial explanation of the D.P. behavior of celluronic acids. If carbonyl groups were removed, the effects of chain scission in alkali could be eliminated and true D.P. of celluronic acids could then be determined. Carbonyls were largely removed by chlorous acid oxidation and sodium borohydride reduction. Although oxidation and reduction of carbonyls resulted in higher alkaline D.P., the D.P. of the modified celluronic acids did not approach the D.P. of unoxidized linters.

On alkaline extraction of celluronic acids, highly oxidized material was preferentially removed, as indicated by the higher carboxyl content and copper number of the soluble fractions. Alkaline extraction also

produced a yellow coloration in the celluronic acids which was not apparent during alkaline extraction of modified celluronic acids. Thus, the oxidation of aldehyde groups to carboxyl groups, or the reduction of aldehyde and ketone groups to hydroxyl groups was also effective in the regions which were overoxidized and alkali soluble. Reduction even decreased the alkali solubility of celluronic acids.

The data suggest that nitrogen dioxide oxidation of cellulose is localized in accessible regions during the early stages. Side oxidation reactions which introduce aldehyde and ketone groups capable of causing chain scission in alkali accompany this localized oxidation of primary hydroxyl groups to carboxyl groups. Furthermore, in the accessible regions, chain scission occurs during oxidation either through acid hydrolysis or overoxidation. It is this degradative effect during oxidation which limits the stabilization of alkaline D.P. on subsequent removal of alkali-sensitive carbonyl groups.

HYDROLYSIS OF CELLURONIC ACIDS

Celluronic acids have been hydrolyzed with 1.0 N hydrochloric acid for periods of 0.5 to 24 hours. The complete data obtained have been tabulated in Table XVIII.

The weight of the hydrolysis residues from celluronic acids and cotton linters have been plotted as a function of the time of hydrolysis in Figure 14. The data for hydrolysis times up to 12 hours are presented in Figure 14. On longer hydrolysis, the residues were discolored with the formation of humic materials (41,42), and the weight losses were

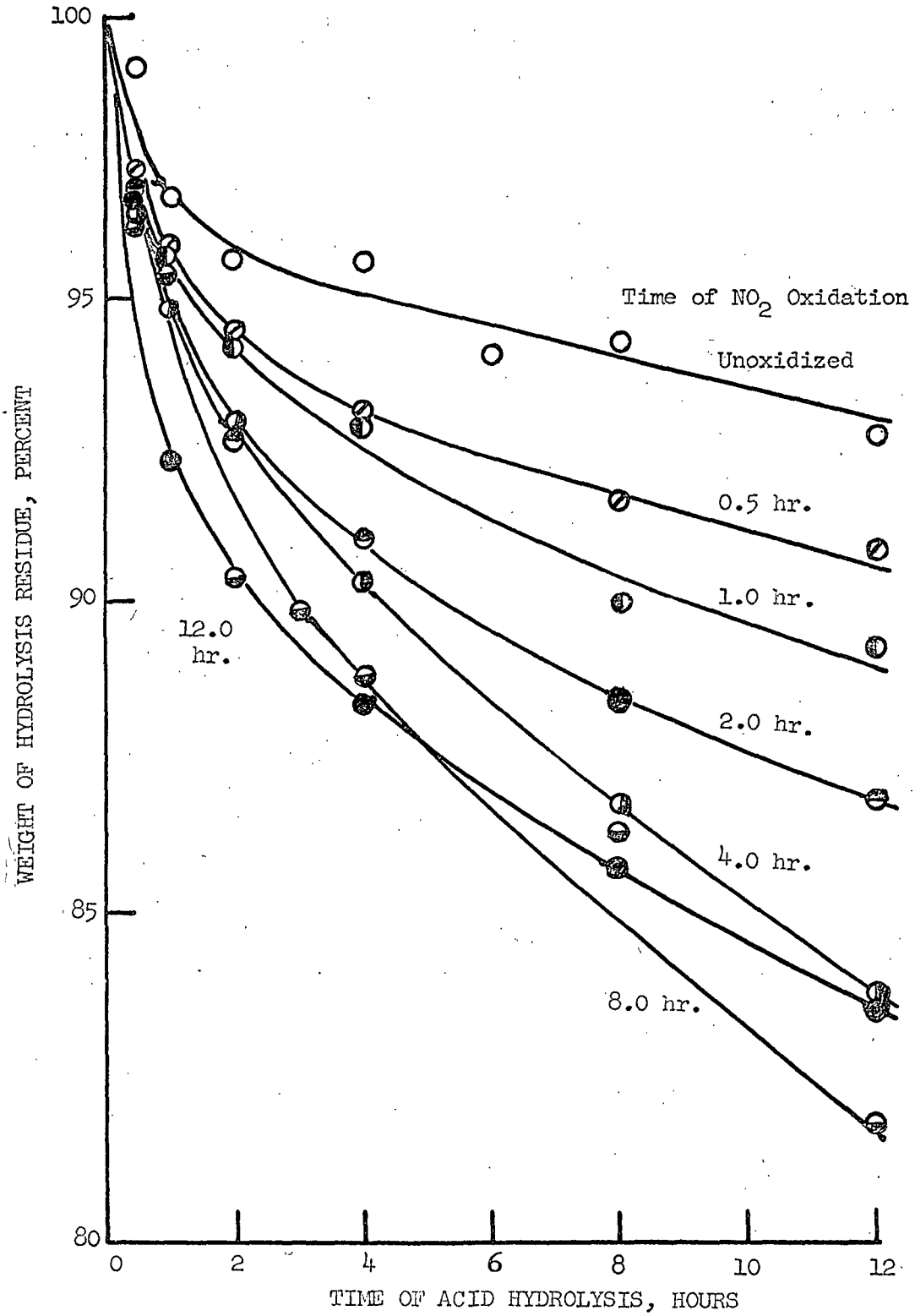

Figure 14

Weight Loss of Celluronic Acids on Acid Hydrolysis

erratic. The humic material formation was greater and occurred earlier during hydrolysis of celluronic acids with increasing degrees of nitrogen dioxide oxidation. This conclusion was based on a qualitative estimation of humic material content evaluated from the discoloration of the residues. An attempt was made to remove the humic materials by extraction with monoethanolamine (42); however, the alkaline monoethanolamine dissolved the greater portion of the celluronic acids and transformed the remainder into a gelatinous mass. The data of Figure 14 were obtained from residues which were relatively free of humic material formation, except for the most highly oxidized celluronic acid.

The rate of hydrolysis and the total amount of material removed on hydrolysis were greater in celluronic acids with increasing levels of oxidation. The greater loss of material has been previously reported; however, the residues of the celluronic acid were not analyzed (16).

Analysis of the residues from the hydrolysis experiments indicated that the leveling-off D.P., a measure of crystallite length, decreases with nitrogen dioxide oxidation. The decrease in the leveling-off D.P. of the celluronic acids is apparent from Figure 15. This lowering in the leveling-off D.P. could be due to a change in crystal structure as noted on mercerization (43,44), to inhibition of recrystallization during hydrolysis by chain scission or reaction of hydroxyl groups in the accessible regions (44,45,46), or to an opening-up of crystallites through penetration of reagents.

X-ray diffraction patterns of hydrolyzed linters, mercerized hydrolyzed linters, and two hydrolyzed celluronic acids are presented

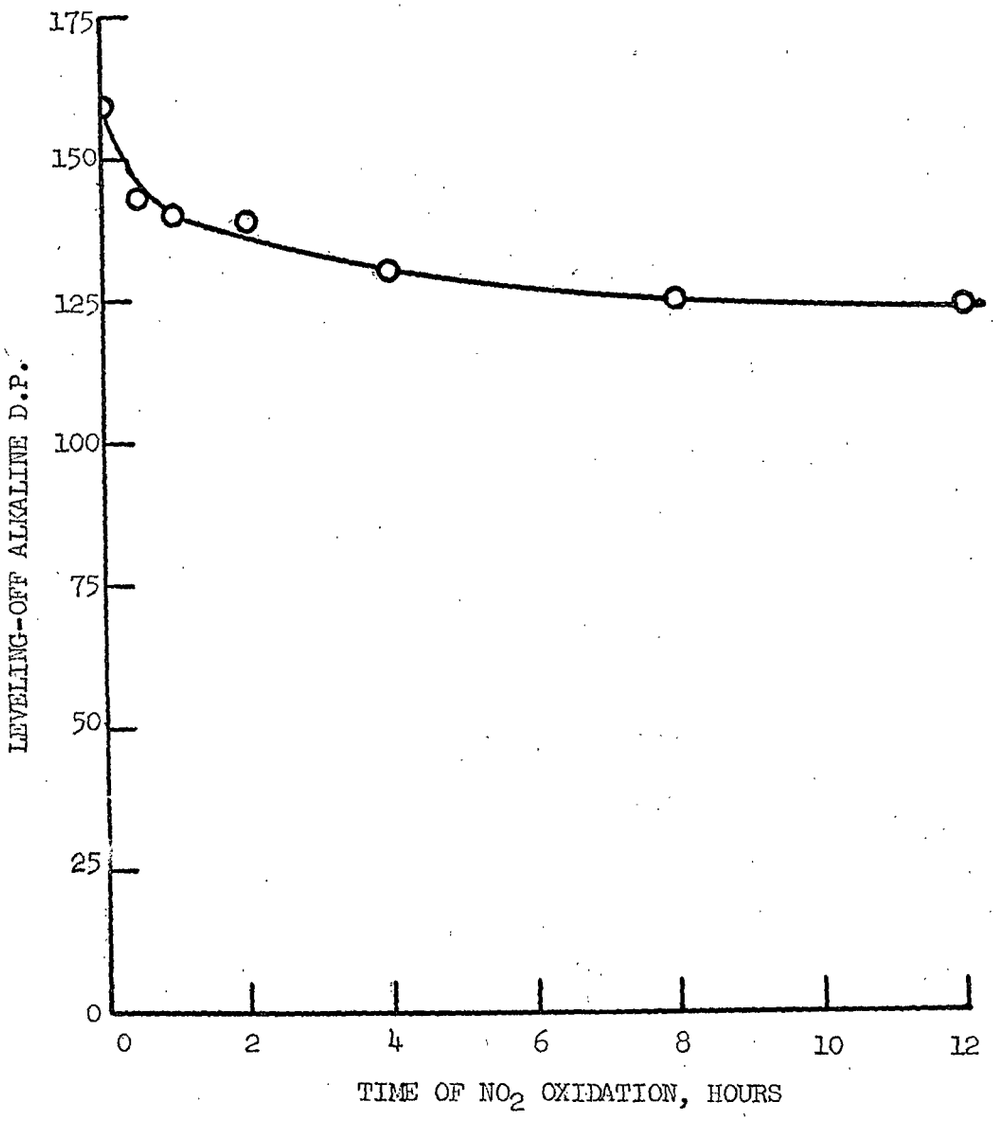

Figure 15

Leveling-off D.P. of Celluronic Acids Found on Acid Hydrolysis

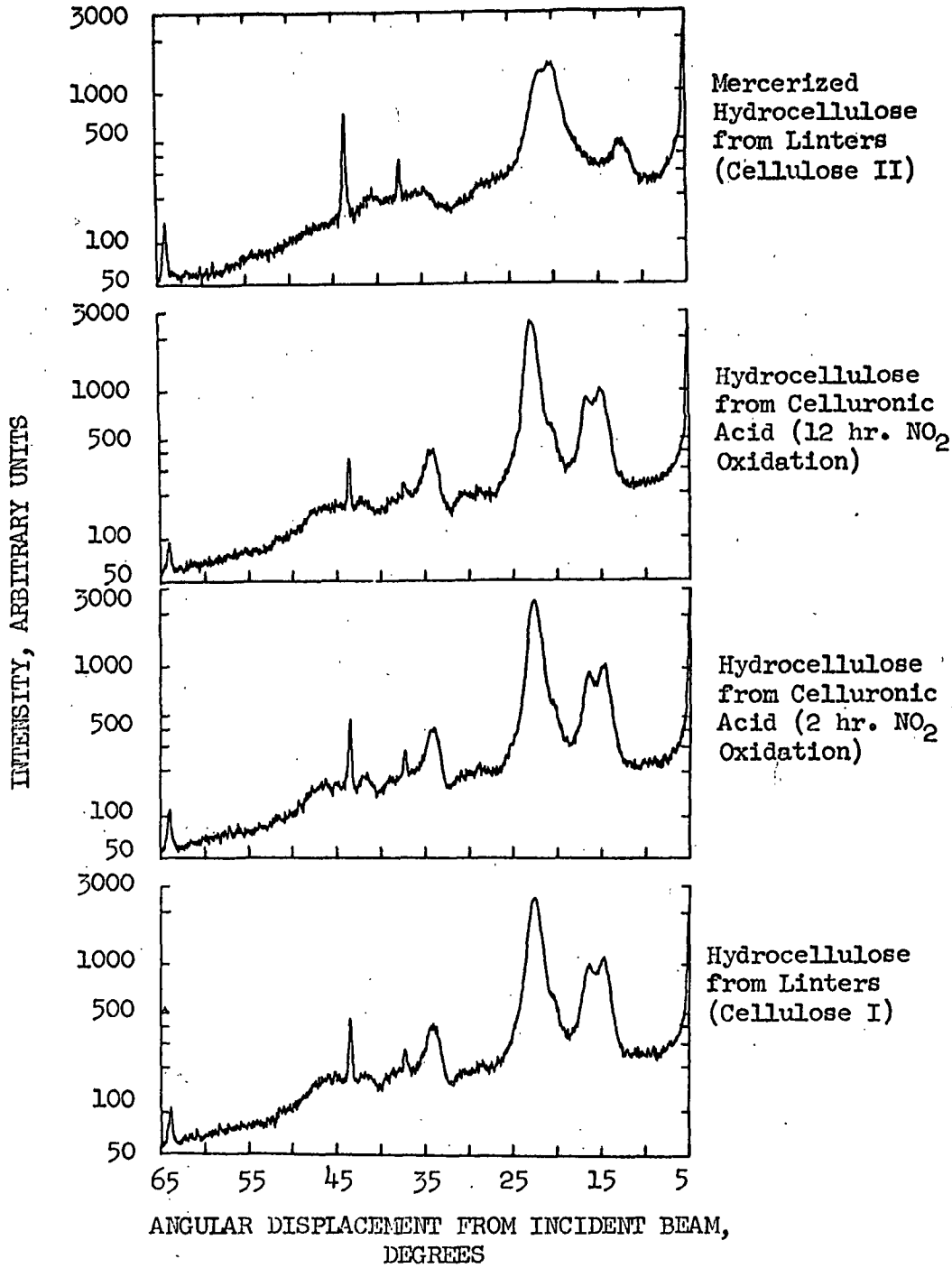

Figure 16

X-ray Diffraction Patterns

for comparison in Figure 16. It is evident from these x-ray diffraction patterns that the crystalline structure of the hydrocelluloses has not been altered by oxidation, and the hydrolyzed celluronic acids retain the structure of Cellulose I. The change in leveling-off D.P. cannot be due to changes in crystal structure. Thus, inhibition of recrystallization on hydrolysis, or an opening-up of crystallites must account for the decrease in leveling-off D.P.

Analysis of the hydrolysis residues from the celluronic acids also indicated that 61 to 65% of the carboxyl groups formed during oxidation were retained after hydrolysis for 24 hours. The carboxyl data for the hydrolysis residues are included in Table XVIII. Since a change in crystal structure is not indicated in Figure 16, the carboxyl groups which were retained by the hydrolyzed celluronic acids must have been located on the surfaces of the crystallites. For the 12-hour nitrogen dioxide oxidation product, the hydrolysis residue analyzed by x-ray retained 2.86% carboxyl. This means that theoretically 11% of the anhydroglucose units present had been oxidized on carbon 6 to carboxyl groups. Based on accepted crystalline dimensions, 19.8% of the primary hydroxyl groups are exposed on the surfaces of the crystallites. This calculation may be found in the Appendix. If other than a surface reaction had occurred, a change in crystal structure should be anticipated with so great an oxidation (47).

The carboxyl content of material removed on 8-hours hydrolysis has been calculated by difference from the data of Table XVIII and plotted in Figure 17 for comparison of the carboxyl contents of the original

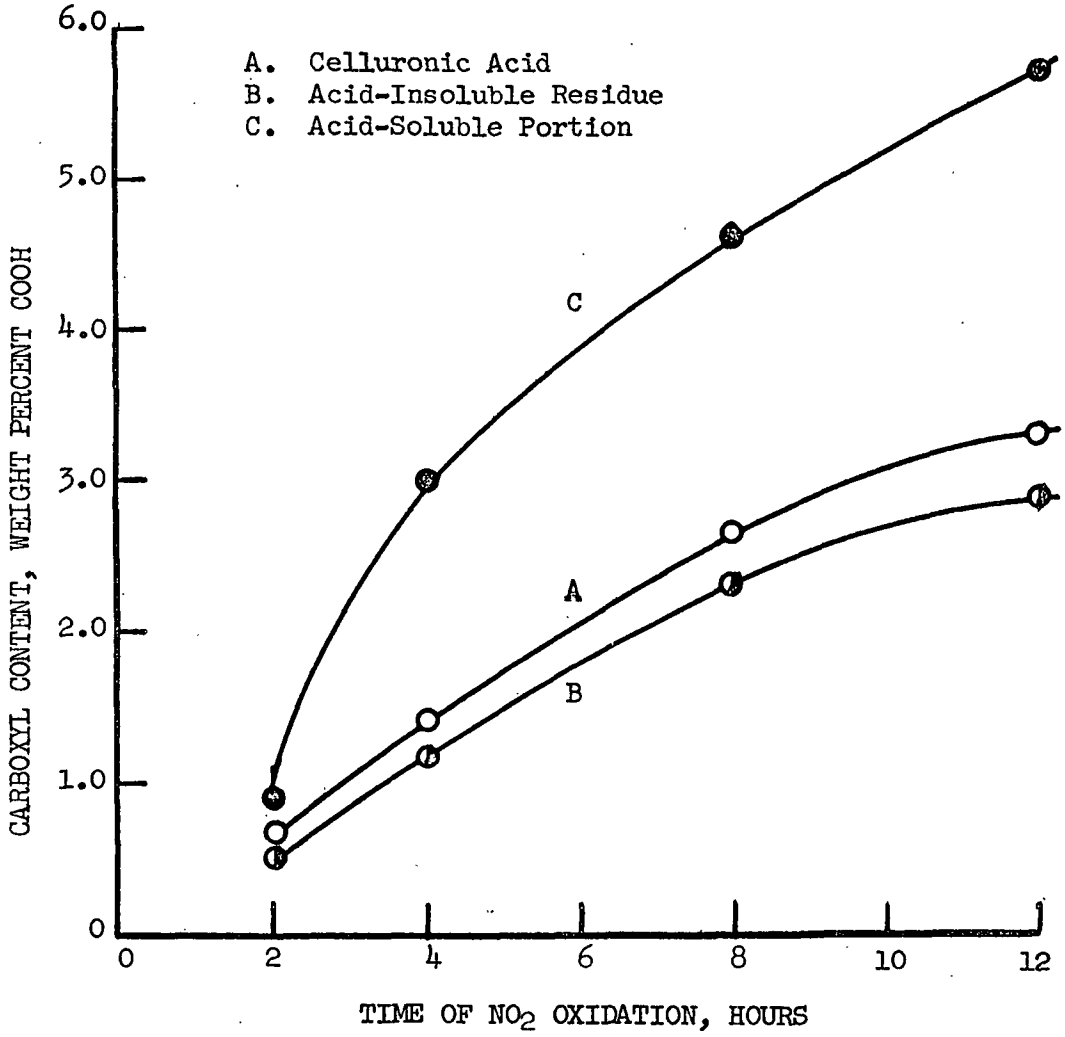

Figure 17

Distribution of Carboxyl Groups after 8 Hours Acid Hydrolysis of Celluronic Acids

celluronic acids and the 8-hour hydrolysis residues. The acid-soluble materials were richer in carboxyl groups than the hydrolysis residues. The 8-hour acid hydrolysis period was selected for illustration in Figure 17 in order to diminish errors in calculations arising from humic material formation. A similar distribution of carboxyl groups occurred for all levels of hydrolysis, however.

POSSIBLE IDENTIFICATION OF GLUCURONIC ACID

After hydrolysis, the acid liquors containing the soluble celluronic acids were combined. The chloride ion was then removed from the hydrolysis liquor by ion exchange with Resin IR-4B (Acetate), and the liquors were concentrated to a small volume. On chromatographic separation in either acetic acid: ethyl lactate: water (2:9:2) or pyridine: butanol: water (3:10:3), the concentrated liquors yielded a spot corresponding to glucuronolactone when the paper chromatograms were sprayed with p-anisidine hydrochloride. The bulk of the concentrated liquor was then chromatographed in the pyridine system and the areas containing the glucuronolactone were removed and eluted with water. The eluate was concentrated, and on addition of absolute ethanol, a few milligrams of precipitate were formed. This precipitate did not melt at temperatures up to 300°C. (glucuronolactone m.p. 167-172°C.). The precipitate did not move when chromatographed in the pyridine system, but gave a spot corresponding to glucuronic acid in the acetic acid system. Apparently sufficient cations were present in the eluent (distilled water) to convert glucuronolactone into a glucuronate. The precipitate gave a positive uronic acid test with naphthoresorcinol (48). A chromatographic analysis of the liquors

from the hydrolysis of the unoxidized cotton linters did not yield a spot corresponding to glucuronolactone.

SUMMARY: ACID HYDROLYSIS

Celluronic acids hydrolyze faster than unoxidized linters in 1.0 N hydrochloric acid at 100°C. After hydrolysis the x-ray diffraction patterns from 2 and 12-hour oxidation products were identical with the diffraction pattern of the hydrolysis residue of cotton linters (Cellulose I). This indicated that no change in crystal lattice occurred during oxidation with a 6% solution of nitrogen dioxide in carbon tetrachloride. It also indicated that these carboxyl groups must have been located on the surfaces of the crystallites.

On oxidation, the leveling-off D.P. of the crystallites from celluronic acids decreased with increasing degrees of oxidation. Presumably this lowering in the crystallite D.P. could have been due to modification of the accessible regions during oxidation with resultant inhibition of recrystallization during hydrolysis, or to pentration of the ends of the crystallites by the oxidizing solution.

The isolation of a material and its possible identification as glucuronic acid offers further evidence that nitrogen dioxide oxidation of cellulose attacks primary hydroxyl groups.

NITRATES OF CELLURONIC ACIDS AND MODIFIED CELLURONIC ACIDS

The celluronic acids and modified celluronic acids were tested for solubility in various nonalkaline solvents before and after nitration.

A summary of the solubility testing may be found in the Appendix. Other than common solvents for cellulose nitrate, i.e., acetone and ethyl lactate, no solvents of superior quality were found for the nitrates of the materials tested. The nitrates of reduced celluronic acids and the nitrates of celluronic acids with less than 0.5% carboxyl were completely soluble in acetone and ethyl lactate. Ethyl lactate solution viscosities were determined on the soluble nitrates and the D.P. was calculated from intrinsic viscosity values by multiplication by the factor 90 (29). These D.P. values are compared with the alkaline D.P. values of reduced celluronic acids in Figure 18 and Table XIX.

Considering the state of the art of D.P. determination by viscosity measurement, the nitrate D.P. values are in good agreement with the alkaline measurements of the reduced celluronic acids. Considerable uncertainty enters the ethyl lactate D.P. calculation through the use of the factor 90 for all samples. The constant for nitrate solutions is dependent on the degree of nitration of the cellulose (49), and, as indicated by the nitrogen contents in Table XIX, the degree of nitration for all samples was not uniform. However, correction of K for variations in nitrogen content does not significantly alter the curve of D.P. for nitrates of reduced celluronic acids in Figure 18.

Included in Table XIX are the nitrogen contents of nitrated celluronic acids which were only moderately swollen in ethyl lactate and acetone. From this data, particularly that for 2 and 12-hour oxidation products, it appears that the degree of nitration alone cannot account for the incomplete solubility of nitrated celluronic acids.

The insolubility of the nitrates of the celluronic acids is apparently

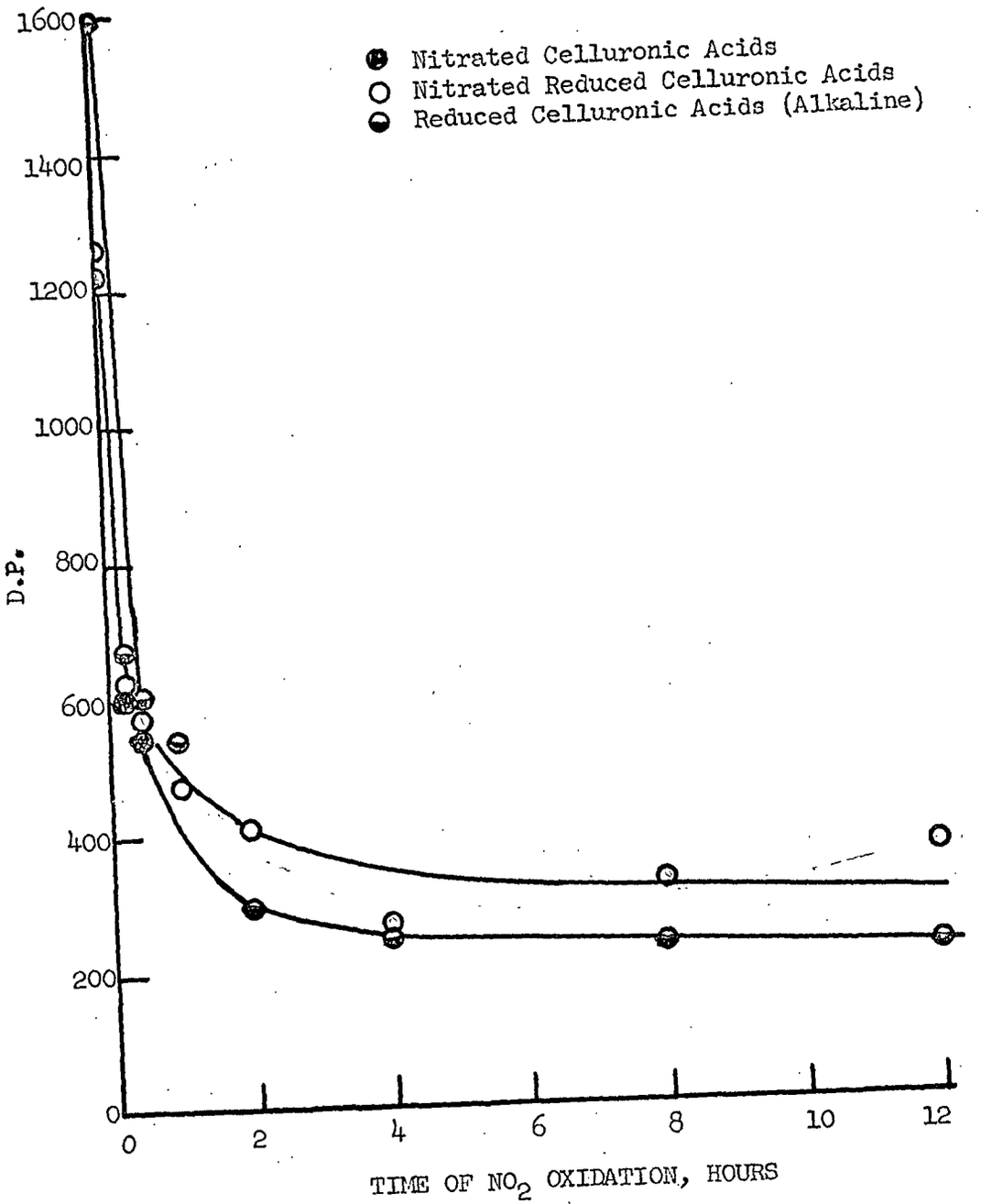

Figure 18

Comparative D.P. Values

related to the carbonyl groups which are introduced by side oxidation reactions. When only the aldehyde groups are oxidized to carboxyl groups with chlorous acid, derived nitrates are still only partially soluble in acetone and ethyl lactate. In periodate-oxidized celluloses, carbonyl groups are present as aldehyde groups, and chlorous acid oxidation of these aldehyde groups leads to nitrates which are soluble in acetone (26). Ketone groups possibly present in celluronic acids differentiate them from periodate-oxidized celluloses.

When the celluronic acids are reduced with sodium borohydride, and ketone groups in addition to aldehyde groups are reduced to hydroxyl groups, the derived nitrates are soluble. Since the reduced celluronic acids are high in carboxyl content, the carboxyl groups either do not affect the solubility of the nitrates or they play a minor role. This theory is in agreement with the solubility of nitrated pectins and celluloses which have been oxidized successively with periodate and chlorous acid. All of these polymers contain carboxyl groups but no carbonyl groups and yield nitrates which are soluble in acetone.

Since the nitrates of linters and celluronic acids with less than 0.5% carboxyl were soluble without further modification, the effects of reduction can be followed. The D.P. values for nitrates of linters and 0.25 and 0.5-hour nitrogen dioxide oxidation products before and after sodium borohydride reduction are in good agreement. The reduction stage did not degrade the linters and celluronic acids. Therefore, it can be concluded from the D.P. data of nitrated reduced celluronic acids that the greatest portion of degradation occurred during the oxidation with

nitrogen dioxide, for the D.P. of these nitrates decreased rapidly during the early stages of oxidation to a leveling-off value (Figure 18).

EXPERIMENTAL RESULTS AND THEIR RELATION TO THE STRUCTURE OF CELLULOSE

The experimental results can be best interpreted if they are related to the structure of cellulose. A widely held concept is that cellulose is a system of polyanhydroglucose chains grouped together in microfibrils which are predominately crystalline, but are periodically interrupted by regions which are not crystalline, i.e., regions of disorder.

The crystalline regions of the cellulose are characterized by their specific x-ray diffraction patterns, by resistance to acid hydrolysis, and by resistance to chromic acid oxidation. The regions of disorder, on the other hand, because of their "open" structure, cause diffraction patterns to be diffuse, and are usually the point of attack of acids and oxidants. The disordered regions can be subjected to chemical reaction for the determination of the amorphous or accessible regions of cellulose. When the hydroxyl groups on the surfaces of the crystallites undergo chemical reaction, the crystallite surfaces are also considered to be included in the accessible regions of the cellulose.

The formation of carboxyl groups and carbonyl groups and the degradation which occurs during oxidation have been related to this concept of cellulose structure in the following discussion.

CARBOXYL FORMATION

Examination of the experimental data leads to the conclusion that carboxyl groups are formed preferentially in the accessible regions of

cellulose on oxidation with nitrogen dioxide up to 12 hours. This conclusion can be arrived at through a consideration of experimental results obtained from analyses of residues remaining after acid hydrolysis and alkaline extraction of the celluronic acids. By necessity, conclusions must be based on the analyses of the residues since the material removed was not available for analysis. The pertinent analyses performed on acid hydrolysis residues are x-ray diffraction patterns, D.P. estimations, and carboxyl estimations.

X-ray diffraction patterns of 12-hour hydrolysis residues from 2- and 12-hour nitrogen dioxide oxidation products are illustrated in Figure 16. These patterns establish the hydrolysis residues as crystalline and of the same structure as the crystallites of unoxidized cotton linters, i.e., Cellulose I.

D.P. determinations in cupriethylenediamine demonstrate that the hydrolysis residues are essentially composed of chains of the same length as those found in crystallites from unoxidized cotton linters. The leveling-off D.P. of celluronic acids from 0.25 to 12-hour oxidation products are illustrated in Figure 15.

Carboxyl assay on the hydrolysis residues and the yield after hydrolysis have been used as a basis for calculation of the distribution of carboxyl groups between the residues and the material which was removed by hydrolysis. These distributions are illustrated in Figure 17. Fewer carboxyl groups accompany the residues than the solubilized material.

From these considerations, it is apparent that the hydrolysis residues

The distribution of carboxyl groups between alkali-extracted residues and alkali-soluble portions of celluronic acids has been calculated from the carboxyl contents of the residues and the yield after extraction. This distribution has been illustrated in Figure 13. The alkali-extracted residues contain fewer carboxyl groups than the alkali-soluble portions.

Thus, the alkali-extracted residues are established as having a base consisting of the crystallites and a carboxyl content which is less than the soluble portion. Therefore, accessible material richer in carboxyl groups than the residues composed the alkali-soluble portion of the celluronic acids. Oxidation of primary hydroxyl groups must have occurred preferentially in these accessible regions.

CARBONYL FORMATION

The possible formation of carbonyl groups during nitrogen dioxide oxidation has been substantiated by copper number (Figure 12), total carbonyl groups as estimated by methylhydroxylamine hydrochloride (Figure 6), and increasing carboxyl contents determined by calcium acetate after chlorous acid oxidation (Table X). All of these measurements indicated increasing carbonyl formation in more extensively oxidized celluronic acids.

On alkaline extraction of the celluronic acids, the reducing groups indicated by copper number were found to be present to a greater extent in the alkali-soluble portions than in the alkali-insoluble residues (Figure 12). This is another indication that oxidation occurred to a greater extent in accessible regions than in crystalline regions.

are crystallites with structure and size similar to the crystallites of unoxidized cellulose. These crystallites have been oxidized to a lesser extent than the material removed during hydrolysis, i.e., non-crystalline or accessible material. Therefore, the carboxyl groups must have been formed preferentially in the accessible regions during nitrogen dioxide oxidation.

The location of the carboxyl groups which are retained by the hydrolysis residues is also of interest. The carboxyl content of the residue from 12-hour oxidation product corresponds to an oxidation of 11% of the primary hydroxyl groups. Ordinarily, this much oxidation would be accompanied by a change in x-ray diffraction patterns if hydroxyl groups within the body of the crystallites has been oxidized. Since the x-ray diffraction pattern of this residue is the same as that of Cellulose I, the residual carboxyl groups must have been formed largely on the surfaces (or ends) of the crystallites (47). According to the definition of accessible regions presented above, the surfaces of crystallites are to be included in accessible regions if the exposed hydroxyl groups undergo oxidation. Therefore, the carboxyl groups retained by the hydrolysis residues also can be considered to be associated regions of the cellulose.

Further evidence of preferential formation of carboxyl groups in accessible regions was obtained on alkaline extraction of the celluronic acids. Again, this conclusion was based on analyses of the residues; in this case, D.P. estimations and carboxyl assays.

D.P. estimations of the extracted residues show that these residues are composed of chains of greater size than crystallites. For this magnitude of D.P. (Figure 8a), these residues must include the crystallites as a base.

In celluronic acids with carboxyl contents ranging from 0.10 to 3.4%, the carbonyl contents, estimated by nitrogen analysis of oximated free acids and expressed as D.S. (Figure 6, Curve 3), have been found to range from 0.009 to 0.032. If these carbonyl groups were distributed randomly between accessible and crystalline regions and if each were a potential source of chain scission, the alkaline D.P. should range from 110 down to 31. These values are lower than the 428 to 170 D.P. values determined for these celluronic acids in cupriethylenediamine. As oxidation continued to 12 hours, the carbonyl content increased while alkaline D.P. reached a leveling-off value (Figures 4 and 8) after oxidation for only 2 hours. Each chain scission would not result in a large decrease in D.P. if the carbonyl groups were localized in the accessible regions of the cellulose.

A higher alkaline D.P. was obtained after aldehyde groups were oxidized to carboxyl groups with chlorous acid, and when ketone and aldehyde groups were reduced to hydroxyl groups with sodium borohydride. However, even with the greater D.P.'s obtained on oxidation and reduction, the final D.P. of the celluronic acids was still much lower than the D.P. of the unoxidized linters. Accordingly, the low D.P. of the celluronic acids in cupriethylenediamine prior to modification is due in part to some other cause than alkali-sensitive groups. This finding tends to contradict Nevell's statements (4) that "The low relative viscosities are not due to scission of chain-molecules during the preparation of the materials" but..."are due to scission resulting from the alkaline nature of the solvent used."

Since the D.P. of unoxidized linters was not obtained after chlorous acid oxidation or reduction of the celluronic acids, some chain scissions

must have occurred during nitrogen dioxide oxidation. These chain scissions could have been caused by oxidations in the accessible regions. With preferential formation of carboxyl and carbonyl groups in the accessible regions, it is not unreasonable to expect this localized oxidation to cause scission of the polyanhydroglucose chains. Furthermore, chain scissions by acid hydrolysis may also be anticipated, since water, a product of oxidation reactions, can form nitric acid, a hydrolytic agent, in the accessible regions by reaction with nitrogen dioxide. The conditions leading to chain scission by either oxidation or acid hydrolysis during nitrogen dioxide oxidation of cellulose are favorable.

DEGRADATION DURING OXIDATION

In alkaline solvents for D.P. determination, degradation during oxidation cannot be differentiated from subsequent chain scissions due to alkali-labile carbonyl groups. Whether or not degradation resulting in chain scission occurred during the nitrogen dioxide oxidation has been determined by measuring the D.P. of celluronic acid nitrates in ethyl lactate, a nonalkaline solvent. Although only the nitrates of celluronic acids produced by 0.25 and 0.5-hour oxidations were soluble in ethyl lactate, the D.P. values (Figure 18) indicated that considerable degradation had occurred upon oxidation for only 0.5 hour.

All the nitrates derived from reduced celluronic acids were found to be soluble in ethyl lactate. The D.P. values determined from the viscosity measurements of ethyl lactate solutions of the nitrates of reduced celluronic acids confirm the rapid degradation of cellulose

during the early stages of nitrogen dioxide oxidation. Since the D.P. of the nitrates also reached a leveling-off value (Figure 18), degradation limited to the accessible regions is indicated.

The possible presence of carbonyl groups, and in particular ketone groups, introduced by side oxidations play an important role in preventing solubility of the nitrates of unreduced celluronic acids. Removal of the aldehyde groups with chlorous acid oxidation was not sufficient to form soluble nitrates. Only after ketone groups and aldehyde groups were reduced to hydroxyl groups did the nitrates of the celluronic acids become soluble. Furthermore, since the reduced celluronic acids retained carboxyl groups, the carboxyl groups do not affect the solubility of nitrates when carbonyl groups are absent.

SUMMARY

The course of the nitrogen dioxide oxidation of cellulose has been interpreted by relating the experimental results to cellulose structure. At the onset, the oxidation is largely localized in the accessible regions. Carboxyl groups are formed through the oxidation of primary hydroxyl groups; carbonyl groups are formed to a lesser extent; and chain scissions occur either by oxidation or by acid hydrolysis at glycosidic linkages. In alkali some further chain scissions occur at carbonyl groups. Since all sources of degradation are limited to accessible regions, the celluronic acids approach a leveling-off D.P. in both alkaline and nonalkaline solvents.

SUMMARY OF EXPERIMENTAL OBSERVATIONS

During the experimental work, the following observations on the properties of celluronic acids have been made in confirmation of previously reported work:

1. The carboxyl content of celluronic acids increases with time of oxidation and the concentration of nitrogen dioxide in carbon tetrachloride.

2. A visual distinction cannot be made between linters and celluronic acids with carboxyl contents up to 14%.

3. The nitrogen content of celluronic acids is low.

4. The alkaline D.P. of celluronic acids falls rapidly from the D.P. of unoxidized linters to a leveling-off value. This property has been previously indicated by relative viscosity measurements on gas phase oxidation products.

5. The copper number of celluronic acids increases with time of oxidation.

6. The alkali solubility of celluronic acids increases with time of oxidation.

7. Chlorous acid oxidation of celluronic acids with up to 3.5% carboxyl causes a loss of yield of 1% or less.

8. The copper number of celluronic acids is decreased by chlorous acid oxidation.

9. Chlorous acid oxidation increases the carboxyl content of celluronic acids.

10. Complete esterification of carboxyl groups in celluronic acids with diazomethane was not obtained.

11. Nitrates of celluronic acids with greater than ca. 0.5% carboxyl are only partially soluble in common organic solvents for cellulose nitrate.

Contrary to previously reported data (16), it has been found that:

12. During the initial stages (0 to 24 hours) of nitrogen dioxide oxidation, the rate of carboxyl formation obtained with nitrogen

dioxide-carbon tetrachloride solutions containing 50% nitrogen dioxide by weight did not conform to a single first order reaction.

Extensions of previously reported work have been made through the following observations:

13. The leveling-off D.P. of the celluronic acids occurs at a value somewhat greater than the D.P. of crystallites.

14. The carboxyl content and D.P. of celluronic acids generally decrease on storage in the free acid form. The decreases are independent of the carboxyl content.

15. Celluronic acids hydrolyze at a faster rate than unoxidized linters.

16. The insoluble nitrates of celluronic acids had levels of nitration comparable to those in nitrates of cellulose and reduced celluronic acids which were soluble in acetone and ethyl lactate.

New data obtained from the characterization and modification experiments on celluronic acids lead to the following observations:

17. Triplicate oxidations of a specific cotton linters with 6% nitrogen dioxide in carbon tetrachloride for 0.25 to 12 hours produced celluronic acids whose carboxyl content varied 0.10% or less at each level of oxidation.

18. The crystalline structure of cellulose is not changed by oxidation with 6% nitrogen dioxide in carbon tetrachloride for 2 or 12 hours.

19. The alkali-soluble portions of celluronic acids with 3.31% carboxyl or less are richer in carboxyl groups than the alkali-extracted residues.

20. The alkali-soluble portions of celluronic acids with 3.31% carboxyl or less are richer in reducing groups than the alkali-extracted residues.

21. Both linters and celluronic acids have lower D.P. after extraction with 0.100 N sodium hydroxide.

22. Chlorous acid oxidation of celluronic acids increases the alkaline D.P. The increase in D.P. is less with more extensively oxidized celluronic acids.

23. Chlorous acid oxidation of celluronic acids with up to 3.06% carboxyl did not change the solubility in 0.100 N sodium hydroxide beyond the limits of experimental error.

24. The yield loss on reduction of celluronic acids with sodium borohydride increases with carboxyl content to 8% in a celluronic acid with 2.98% carboxyl.

25. The copper number of celluronic acids is lowered by reduction.

26. In celluronic acids, the majority of the carboxyl groups estimated with calcium acetate are not affected by sodium borohydride reduction.

27. The alkaline D.P. of celluronic acids is increased by sodium borohydride reduction. The extent of the increase diminishes with more extensively oxidized celluronic acids.

28. The alkali solubility of celluronic acids is decreased by reduction. The decrease in alkali solubility is appreciable even after correction for yield losses during reduction.

29. A yellow color forms when celluronic acids are treated with alkali. The color bodies are alkali soluble.

30. Celluronic acids oxidized by chlorous acid or reduced by sodium borohydride do not produce a yellow color when treated with alkali.

31. The leveling-off D.P. of crystallites determined by acid hydrolysis is decreased by nitrogen dioxide oxidation.

32. The rate of humic substance formation on hydrolysis of celluronic acids increases with carboxyl content.

33. On acid hydrolysis, removal of easily hydrolyzed material does not remove all carboxyl groups.

34. The material removed by acid hydrolysis from celluronic acids with carboxyl contents of 3.31% or less is richer in carboxyl groups than the hydrolysis residues.

35. A material was isolated from the hydrolysis liquors of celluronic acids and possibly identified as glucuronic acid.

36. Carboxyl groups retained by hydrolysis residues do not alter x-ray diffraction patterns.

37. Diazomethane esterification of celluronic acids does not improve the solubility of derived nitrates in common organic solvents for cellulose nitrate.

38. Chlorous acid oxidation of celluronic acids does not improve the solubility of derived nitrates in common organic solvents for cellulose nitrate.

39. Nitrates of reduced celluronic acids are soluble in common organic solvents for cellulose nitrate.

40. Nitrates of celluronic acids with low carboxyl contents (soluble in ethyl lactate without modification) have a lower D.P. than nitrates of unoxidized cellulose.

41. The D.P. of nitrates of reduced celluronic acids fall rapidly to a leveling-off value with increasing carboxyl contents in the celluronic acids.

CONCLUSIONS

The following conclusions have been derived from new data presented in this thesis:

Conditions for the oxidation of cellulose with nitrogen dioxide in carbon tetrachloride were established which gave a reproducibility of ± 0.05% in carboxyl content.

The isolation of a material and its possible identification as glucuronic acid is the first direct evidence that carbon atom 6 of anhydroglucose units in cellulose is involved in the formation of carboxyl groups on nitrogen dioxide oxidation.

From the onset, nitrogen dioxide oxidation occurs preferentially in the accessible regions, for, when the accessible regions were solubilized either by acid hydrolysis or alkaline extraction, they were found to be richer in carboxyl groups than the residues.

From the yellow color of the oxidized materials which are soluble in dilute alkali, it is inferred, by analogy to known chromophores, e.g., α-diketones, that localized overoxidation has occurred.

The reduction of most (80%) of the carbonyl groups by sodium borohydride did not proportionately restore the D.P. of the celluronic acids to that of unoxidized cellulose; consequently, their low D.P. is not due entirely to the sensitivity to alkali of carbonyl groups which may be present.

The same conclusion holds for the alkali-sensitive aldehyde groups which were specifically oxidized by chlorous acid.

The reaction with sodium borohydride is not responsible for the low D.P. of celluronic acids because the nitrates of celluronic acids with less than ca., 0.5% carboxyl, which have never come in contact with alkali, exhibit the same D.P. in ethyl lactate as the nitrates of the reduced celluronic acids.

The residues obtained from celluronic acids by acid hydrolysis are crystallites with the structure of Cellulose I, as shown by the x-ray diffraction patterns, and the carboxyl groups retained by these residues probably are located on the surfaces of the crystallites.

These crystallites remaining after acid hydrolysis have about the same average length as the crystallites from cellulose because they have about the same leveling-off D.P.

The insolubility of the nitrated celluronic acids (carboxyl>0.5%) in the usual solvents for cellulose nitrate can be attributed to the influence of carbonyl groups, particularly ketone groups, because their reduction with sodium borohydride resulted in soluble nitrates, whereas oxidation of aldehyde groups or esterification of carboxyl groups did not lead to soluble nitrates.

The insolubility of nitrated celluronic acids is not the result of lack of nitration where carbonyl groups occur, because the insoluble nitrates had levels of nitration corresponding to those in soluble nitrated-celluloses and soluble nitrated-reduced-celluronic acids.

The net interpretation of all of these specific conclusions is that degradation of cellulose occurs during the reaction with carbon

tetrachloride solution of nitrogen dioxide because of localized oxidation or acid hydrolysis and that the low D.P.'s observed are not due entirely to the presence of alkali-sensitive carbonyl groups in otherwise intact chains.

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Nitrogen dioxide alone was found to oxidize cellulose to form products soluble in hot dilute sodium carbonate. No nitration products were formed unless considerable amounts of nitric acid were present.

2. Yackel, E. C., and Kenyon, W. O. Oxidation of cellulose with nitrogen dioxide. J. Am. Chem. Soc. 64:121 (1942).

Cellulose was oxidized with gaseous nitrogen dioxide by both the static and cyclic methods for production of a material high in carboxyl content. The carboxyl content was estimated by titration of excess alkali added to the sample, by the calcium acetate method, and by carbon dioxide evolution. The calcium acetate and carbon dioxide evolution methods were in agreement, but the titration of excess alkali gave variable results depending on the time of exposure of the sample to the alkali.

The oxidation products were fibrous even at carboxyl contents of 15%, and had a high affinity for water and basic dyes. Cellouronic acids with greater than 13% carboxyl dissolved completely in aqueous dilute alkalies, while materials of lower carboxyl formed swollen gelatinous masses. No nitrogen was found in the products within the limits of the micro-Dumas method.

3. Unruh, C. C., and Kenyon, W. O. Investigation of the properties of cellulose oxidized by nitrogen dioxide, I. J. Am. Chem. Soc. 64: 127 (1942).

Cyclic gaseous oxidation of cellulose with nitrogen dioxide gave products with copper numbers which increased with degree of

oxidation. A limiting value of 25% carboxyl was reached. The carboxyl groups were assumed to be located on carbon 6.

4. Nevell, T. P. The oxidation of cotton cellulose by nitrogen dioxide. J. Textile Inst. 42, no. 3:T91 (1951).

This paper is an extensive work on the properties of celluronic acids with a wide range of carboxyl contents which were produced by the static gaseous nitrogen dioxide oxidation of cellulose. The relative breaking strength of oxidized yarns fell rapidly in the early stages of oxidation to 25% of the original strength at a carboxyl content of about 1%. Further increases in the carboxyl content resulted only in a slow additional decrease in the relative breaking strength. Relative viscosities of the celluronic acids in alkaline solvents fell from the high values (10 to 100) of unoxidized cellulose to around 1.5 (for 0.5 gram celluronic acid per 100 ml. of cuprammonium) at a carboxyl content of about 2.5%. However, at greater carboxyl contents, little further decrease in relative viscosity occurred. The decreases in viscosity were attributed to chain scission in the alkaline solvent.

The fibrous nature of the products, the inability to achieve complete regeneration of celluronic acids from alkali, the high copper number, and the increase in carboxyl content and decrease in copper number on chlorous acid oxidation were used to support the theory of the formation of alkali-sensitive carbonyl groups by side reactions. Celluronic acids with greater than 9% carboxyl were completely soluble in 0.1 N sodium hydroxide at 20°C. The calcium acetate method was employed in the determination of carboxyl groups. Oxidations beyond the theoretical maximum were obtained.

The nitrates of the celluronic acids containing as low as 1.5 millimoles of carboxyl per 100 grams were found to be only partially soluble in acetone.

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Nitrogen dioxide in carbon tetrachloride was used for the production of celluronic acids in a study of the reaction mechanism. The mechanism proposed is the formation of a mononitrate of cellulose with the nitrate located in the 6 position. This reaction is then followed by a series of slower unknown reactions leading to denitration and the formation of a carboxyl group. Nitrogen dioxide appears to be essential in limiting nitration to the mononitrate and in converting the nitrate to a carboxyl.

7. Maurer, K., and Drefahl, G. Oxidation with nitrogen dioxide, I. Preparation of glyoxylic acid, glucuronic acid, and galacturonic acid. Ber. 75B: 1489 (1942); C.A. 38:1210.

Oxidation of galactose with nitrogen dioxide yielded mucic acid. Oxidation of alpha-methyl glucoside and alpha-methyl galactoside yielded the corresponding uronic acids. These oxidations indicated the preferential attack of primary hydroxyl groups by nitrogen dioxide.

8. Maurer, Kurt, and Reiff, Georg. Oxidations of cellulose with nitrogen dioxide. J. Makromol. Chem. 1, no. 1/6; 27 (1943).

That preferential attack of nitrogen dioxide on cellulose is the oxidation of primary hydroxyl groups to carboxyl groups is proven by the ease of splitting off carbon dioxide from celluronic acids with hydrochloric acid. Unimportant decomposition occurring during the oxidation is attributed to side reactions which cause instability in alkali, as noted by increasing copper number.

9. Head, F. S. Formation of tartaric acid by oxidation and hydrolysis of oxycelluloses produced by dinitrogen tetroxide. J. Chem. Soc. 1948: 1135 (Aug., 1948).

Evidence supporting the nitrogen dioxide oxidation of primary hydroxyl groups to carboxyl groups was obtained by oxidation of a celluronic acid with metaperiodate followed by chlorous acid. This oxidized product was then hydrolyzed and mesotartaric acid isolated.

10. McGee, P. A., Fowler, W. F., Jr., Unruh, C. C., and Kenyon, W. O. Investigation of the properties of cellulose oxidized by nitrogen dioxide, VI. The effect of alkali on the celluronic acids. J. Am. Chem. Soc. 70:2700 (1948).

Investigation of celluronic acids produced by oxidation with solutions of nitrogen dioxide in carbon tetrachloride lead to the conclusion that side oxidation forming alkali-labile linkages accompany carboxyl formation. Small amounts of carbonyl groups were found by the methylhydroxylamine method and could account for the consumption of alkali and the high copper numbers of celluronic acids. It is hypothesized that these carbonyl groups also account for the decreases in viscosity noted by other investigators.

The calcium acetate method for carboxyl determination was

found to give reliable results at equilibrium pH values as low as 5.4.

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Chromic acid oxidation of cellulose is accompanied by a continuous decrease in weight and tensile strength. At high degrees of oxidation the material falls to a powder without strength, x-ray diffraction patterns remain unchanged, and fluidity and copper number values reach leveling-off values. Apparently highly oxidized materials become soluble, leaving crystallites behind with only minor attack by the oxidant. The nitrates of these oxidized celluloses at all degrees of oxygen consumption are soluble in acetone.

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Oxidation of cellulose with hypobromite produces materials whose strength decreases as the product falls to a powder. The x-ray diagrams of the oxidized products are essentially unchanged by oxidation. The fluidity and copper number rapidly increase to leveling-off values as oxidation proceeds.

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A high proportion of the primary hydroxyl groups of cellulose can be oxidized to carboxyl groups with nitrogen dioxide without destroying the crystalline structure of cellulose. Very highly oxidized materials gave diffuse x-ray diagrams. These results lead to the conclusion that nitrogen dioxide is the oxidizing species in gaseous oxidations.

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Solutions of high concentration of nitrogen dioxide in carbon tetrachloride (50:50) are capable of swelling the crystallites of cotton linters as evidenced by the x-ray pattern of regenerated cellulose obtained after a treatment too short to produce significant oxidation. In more dilute solutions of nitrogen dioxide in carbon tetrachloride, the amorphous regions appear to be oxidized preferentially, as interpreted from extrapolation of curves of percentage of anhydroglucose units unoxidized vs. time of oxidation. A celluronic acid with a carboxyl content corresponding to complete oxidation of the amorphous regions lost a greater amount of weight on acid hydrolysis than unoxidized cellulose.

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By measuring nitrate fluidities on hydrocelluloses and oxidized celluloses it has been shown that celluloses oxidized in acid or neutral medium exhibit a decrease in D.P. after alkaline treatment. The fluidity of hydrocelluloses and celluloses oxidized in alkaline mediums were not affected by an additional alkaline treatment. Cuprammonium fluidities were not altered by the alkaline treatments, indicating the degradation was inherent in alkaline viscosity measurements.

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Regenerated cellulose oxidized with nitrogen dioxide showed properties of high alkali solubility, increased copper number, and decreased alkaline D.P.

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A method is described for the determination of carbonyl groups with methylhydroxylamine hydrochloride solution. The reaction was found to be essentially complete in 24 hours. Methylhydroxylamine hydrochloride offers the advantage of reagent stability.

The calcium acetate method was found superior to the silver o-nitrophenolate method for determining carboxyl contents of reducing oxidized celluloses. A reaction time exceeding 15 hours was required for complete reaction of calcium acetate with some oxidized celluloses.

A procedure is given for the chlorous acid oxidation of oxidized celluloses.

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Aldoses are oxidized to the corresponding aldonic acids with acidic sodium chlorite solutions. Ketoses, aldonic acids, and polyhydroxy alcohols are unaffected by this reagent. Chlorous acid was found to be the oxidant.

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In studies on periodate-oxidized celluloses, chlorous acid was employed for oxidizing aldehyde groups to carboxyl groups. Nitrocelluloses from samples which had consumed more than 0.05 millimole of periodate per gram of cellulose were not completely soluble in acetone until the aldehyde groups had been oxidized to carboxyl groups with chlorous acid.

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28. Feigl, Fritz. Spot tests. II. Organic applications, p. 126. Amsterdam, Elsevier Publishing Co., 1954.

Organic nitrites and nitrates give a blue color with diphenylamine.

29. Gloor, W. E., and Klug, E. D. In Cellulose and cellulose derivatives. 2nd. ed. Vol. III. p. 1440. New York, Interscience Publishers, Inc., 1955.

For calculation of D.P. from intrinsic viscosities from the equation D.P. = K [n], the values of K = 150 for cellulose in cupriethylenediamine and K = 90 for cellulose nitrate in ethyl lactate are recommended.

30. Luff-Schoorl method for copper number. In Polarimetry, saccharimetry and the sugars. Circular C 440. U. S. Dept. Commerce, National Bureau of Standards. p. 195. Washington, U. S. Government Printing Office, 1942.

31. Kjeldahl nitrogen with hydriodic acid. In Official methods of analysis of the Association of Official Agricultural Chemists. 8th. ed. p. 728. Washington, Association of Official Agricultural Chemists, 1955.

32. Davidson, G. F., and Standing, H. A. Auto-hydrolysis of acidic oxycelluloses. J. Textile Inst. 42, no. 3:T141 (1951).

Acidic celluloses produced by hypobromite oxidation and successive periodate and chlorous acid oxidation were found to increase in fluidity and decrease in carboxyl on storage in the free acid form. Auto-hydrolysis has been suggested as a hypothesis for explaining these changes.

33. Davidson, G. F. Progressive oxidation of cotton cellulose by periodic acid and metaperiodate over a wide range of oxygen consumption. J. Textile Inst. 32:T109 (1941).

After initial rapid increase during early stages of oxidation, fluidity and copper number continue to increase at a slower rate throughout oxidation of cellulose with periodic acid and metaperiodate. The limiting value of fluidity is imposed by the fluidity of the cuprammonium solvent.

Nitrates of the oxidized celluloses are incompletely soluble in acetone after 0.02 atom oxygen per glucose unit has been consumed during oxidation. Insolubility is not due to low nitrogen content in the nitrates.

34. Speiser, R., and Eddy, C. R. Effect of molecular weight and method of deesterification on the gelling behavior of pectin. J. Am. Chem. Soc. 68:287 (1946).

Acetone was used as a solvent for nitrated pectins in the determination of D.P.

35. Schlubach, H. H., and Hoffmann-Walbeck, H. P. On native pectin. Makromol Chem. 4:5 (1949).

Nitrated pectins with as low as 9% of the theoretical maximum methoxyl content were suitable for D.P. determinations in acetone.

36. Head, F. S. The action of diazomethane on cotton and various modified cotton celluloses. J. Textile Inst. 43:T1 (1952).

The carboxyl groups of nitrogen dioxide-oxidized celluloses were esterified when the celluronic acids were treated with anhydrous diazomethane-ether solutions at 0°C. When water was added, etherification occurred in addition to esterification.

Methylated celluronic acids did not dissolve completely in cuprammonium. No evidence of stabilization of celluronic acids to alkali was found on diazomethane treatment, although copper number was reduced somewhat.

37. Reeves, R. E. Stabilization of oxidized cotton fiber. Ind. Eng. Chem. 35:1281 (1943).

Treatment of periodate-oxidized celluloses with diazomethane and chlorous acid stabilized them to alkali. Reaction of diazomethane with aldehyde groups was offered as an explanation of stabilizing action, although confusion arose through simultaneous reaction of diazomethane with certain hydroxyl groups.

38. Head, F. S. The reduction of the aldehyde groups in periodate oxycelluloses by sodium borohydride. J. Textile Inst. 46:T400 (1955).

Periodate-oxidized celluloses were stabilized to alkali by sodium borohydride reduction. Stabilization was indicated by decreased copper number, fluidity, and alkali solubility. The greatest decreases in these properties were obtained with unbuffered sodium borohydride solutions at 20°C. The reaction was complete in 24 hours. The fluidity decrease obtained on reduction was greater than that obtained by chlorous acid oxidation of the oxidized celluloses.

39. Head, F. S. The reduction of aldehyde groups in hydrocelluloses by sodium borohydride. J. Textile Inst. 46:T584 (1955).

Reduction of aldehyde groups in hydrocelluloses with sodium borohydride was found to be less difficult than reduction of aldehyde groups in periodate-oxidized celluloses. Virtually all aldehyde groups could be reduced, and the hydrocelluloses stabilized to alkali. Copper number and loss of weight on boiling with dilute alkali were diminished to very low values with an unbuffered sodium borohydride solution at 20°C.

40. Chaikin, S. W., and Brown, W. G. Reduction of aldehydes, ketones, and acid chlorides by sodium borohydride. J. Am. Chem. Soc. 71:122 (1949).

Acids and acid anhydrides show only slight reduction with sodium borohydride. This milder reducing property of sodium borohydride compared to lithium aluminum hydride is advantageous in the selective reduction of carbonyl groups in the presence of functional groups (free carboxyl groups) which would also be reduced with lithium alumium hydride.

41. Philipp, H. J., Nelson, M. L., and Ziifle, H. M. Crystallinity of cellulose fibers as determined by acid hydrolysis. Textile Research J. 17:585 (1947).

The formation of humic materials on hydrolysis is discussed. The humic materials are insoluble in hydrolyzing acids and introduce an error into the weights of the residues. Presumably these humic substances were formed on polymerization of glucose dehydration products. A correction for humic material formation from glucose in 2.5, 4, and 6 N hydrochloric acid at 100°C. is proposed.

42. Nelson, M. L., and Conrad, C. M. Improvements in the acid hydrolysis method of determining the crystallinity of cellulose. Textile Research J. 18:149 (1948).

The removal of humic substances from hydrolysis residues before

weighing was accomplished with 50% monoethanolamine in a 5-minute extraction at 110 to 115°C.

43. Davidson, G. F. The rate of change in the properties of cotton cellulose under the prolonged action of acids. J. Textile Inst. 34:T87 (1943).

Mercerization of cotton cellulose reduced the leveling-off D.P. from 190 to 60.

44. Roseveare, W. E. Characterization of cellulose by hydrolysis. Ind. Eng. Chem. 44:168 (1952).

Mercerization of cotton linters and wood pulps produced a large decrease in leveling-off D.P. obtained on hydrolysis. In addition to changing the crystal lattice, mercerization also changes the network of crystallites to a new one with much shorter periodicity.

Recrystallization on hydrolysis was largely eliminated by dichromate oxidation of accessible regions. The D.P. of linters after hydrolysis was 190. After oxidation and hydrolysis the D.P. dropped to 149. This hypothesized that the D.P. of crystallites is that which is obtained after oxidation and hydrolysis.

45. Howsman, J. A. Water sorption on the poly-phase structure of cellulose fibers. Textile Research J. 19:152 (1949).

High values of crystalline content of cellulose are reported to be obtained from high temperqture acid hydrolysis experiments. Through the use of water sorption and x-ray diffraction patterns, these high values were attributed to recrystallization of disordered regions on hydrolysis.

46. Battista, O. A. Hydrolysis and crystallization of cellulose. Ind. Eng. Chem. 42:502 (1950).

The basic or leveling-off D.P. of various celluloses were found

to be dependent on conditions of hydrolysis. Mild conditions resulted in a greater amount of recrystallization during hydrolysis and a greater D.P. than obtained with drastic hydrolysis conditions.

47. Clark, G. L. Personal communication, 1956.

If the formation of carboxyl groups is restricted to the surfaces of the crystallites, the surface layer of reacted polyanhydroglucose units would not be of sufficient thickness or order to give a separate diffraction pattern. This would account for a lack of variation in the x-ray diffraction patterns.

48. Tollens, B., and Rorive, F. Color and spectral reactions of sugars with naphthoresorcinol and hydrochloric acid. Ber. 41:1783 (1908); C.A. 2:2693.

After heating of uronic acids with naphthoresorcinol in hydrochloric acid, a blue color can be extracted with ethanol.

49. Doty, P. M., and Spurlin, H. M. In Cellulose and cellulose derivatives. 2nd. ed. Vol. III. p. 1168. New York, Interscience Publishers, Inc., 1955.

A comparison of K values for nitrocelluloses of varying nitrogen content is given when acetone solution viscosities are used in D.P. estimations.

50. McGee, P. A., Fowler, W. F., Jr., and Kenyon, W. O. Investigation of the properties of cellulose oxidized by nitrogen dioxide, III. The reaction of carboxyl groups of polyuronides with calcium acetate. J. Am. Chem. Soc. 69:347 (1947).

The calcium acetate method of carboxyl determination was investigated through carboxyl determinations on celluronic acids and natural polymers. The values obtained varied somewhat from carboxyl contents determined by carbon dioxide evolution. The reaction period did not effect carboxyl estimations beyond experimental error.

51. Arndt, F. In Organic synthesis. Coll. Vol. II. p. 165. New York, John Wiley and Sons, Inc., 1943.

A procedure is given for the preparation of diazomethane from nitrosomethylurea.

52. Arndt, F. In Organic synthesis. Coll. Vol. II. p. 461. New York, John Wiley and Sons, Inc., 1943.

The synthesis of nitrosomethylurea from methylamine hydrochloride and urea is described.

53. Institute Method 18. Methoxyl groups in wood. 1951.

54. Institute Method 421. Alpha-cellulose in pulp. 1951.

55. Institute Method 425. Beta- and gamma-cellulose in pulp. 1951.

56. Browning, B. L., Sell, L. O., and Abel, W. Cellulose solvents for viscosity measurements. Tappi 37, no. 7:273 (1954).

An apparatus is described for the preparation of viscosity samples under nitrogen.

57. Mitchell, R. L. Precipitation fractionation of cellulose nitrate. Ind. Eng. Chem. 45, no. 11:2526 (1953).

A nondegrading method for nitration of cellulose using 90% nitric acid and phosphoric anhydride is given.

58. Shaefer, W. E., and Becker, W. W. Determination of nitrogen in nitrocellulose and nitrocellulose and combined phthalate in lacquers. Anal. Chem. 25:1226 (1953).

The nitrate content of cellulose nitrates is determined by the oxidation of ferrous chloride to ferric chloride. The excess ferrous chloride used in the reaction is then oxidized with titanous chloride.

59. McBurney, L. F. In Cellulose and cellulose derivatives, 2nd. ed. Vol. I. p. 133. New York, Interscience Publishers, Inc., 1954.

60. Howsmon, J. A., and Sisson, W. A. In Cellulose and cellulose derivatives. 2nd. ed. Vol. I. p.237. New York, Interscience Publishers, Inc., 1954.

61. Ströle, U. Determination of carbonyl groups in oxidized celluloses. Makromol. Chem. 20, no. 1:19 (April, 1956).

When sodium borohydride was used as a method of carbonyl determination in celluronic acids, the same carbonyl content was found in solutions whose pH varied from 9 to 11.5. The carbonyl groups found by the sodium borohydride method were in agreement with those estimated by reaction of hydroxylamine with the sodium salts of the celluronic acids.

APPENDIX

EXPERIMENTAL DATA

TABLE I

PRELIMINARY OXIDATIONS
CARBOXYL CONTENTS OF CELLURONIC ACIDS

(90 grams nitrogen dioxide-carbon tetrachloride solution per gram of cellulose)

Time of Oxidation, Hours	Concentration NO_2 in CCl_4, Wgt. % 3	6	12	25	50
	COOH, %	COOH, %	COOH, %	COOH, %	COOH, %
0.5	0.21	0.25	0.23	0.45	0.64
1.0	0.41	0.43	0.58	0.87	1.36
2.0	0.57	0.78	1.25	2.35	3.10
4.0	1.12	1.43	2.36	3.69	5.52
8.0	1.92	2.75	3.79	5.92	9.10
16.0	2.82	4.17	5.25	8.24	12.38
24.0	3.44	5.08	7.00	10.85	14.68

TABLE II

EFFECT OF OXIDIZING MEDIUM TO CELLULOSE RATIO

Time of Oxidation, Hours	NO_2-CCl_4/Linters Ratio, g./g.	Concentration of NO_2 in CCl_4, Wgt. %	
		3	25
		COOH, %	COOH, %
2.0	50	0.42	2.74
	90	0.57	2.35
	135	0.51	3.00
12.0	50	2.27	10.16
	90	2.28	9.71
	135	2.34	9.80

TABLE III

PROPERTIES OF CELLURONIC ACIDS

NO_2 Oxidation Time, Hours	Cotton Linters O.D.F.**	Oxidized Sample	Carboxyl Content, Wgt. %	Ash, Wgt. %	Dumas Nitrogen*, Wgt. %	Alkaline D.P. [d] x 150
0		Linters	0.01	0.11	0.12	1600
0.25	96.3	A	0.10	0.11	0.28	428
		B	0.10	0.02		434
		C	0.10	0.02		446
0.5	96.3	A	0.20	0.04	0.44	373
		B	0.16	0.04		367
		C	0.19	0.19		374
1.0	96.3	A	0.31	0.05	0.45	320
		B	0.37	0.10		320
		C	0.39	0.05		300
2.0	95.9	A	0.77	0.04	0.24	217
		B	0.76	0.04		237
		C	0.76	0.03		222
4.0	95.9	A	1.48	0.04	0.24	215
		B	1.56	0.04		222
		C	1.57	0.04		243
8.0	96.4	A	2.71	0.03	0.24	222
		B	2.68	0.03		243
		C	2.72	0.06		229
12.0	96.4	A	3.36	0.04	0.24	229
		B	3.32	0.05		220
		C	3.46	0.10		201

*Dumas nitrogen determinations made by the Analytical Department.
**Oven Dry Factor, fraction nonvolatile on drying at 105°C. for 16 hrs.

TABLE IV

ALKALI SOLUBILITY OF COTTON LINTERS AND CELLURONIC ACIDS

Time Oxidation, Hours	Oxidized Sample	Alkali Solubility, -Wgt. % From Residue Wgt. A	B	Av.	From Filtrate Oxidations* A	B	Av.
0-linters		0.9	0.8	0.8	--	--	--
0.5	A	1.8	1.6	1.7	1.3	1.4	1.4
1.0	A	2.5	2.1	2.3	2.0	2.0	2.0
2.0	A	4.0	4.0	4.0	4.8	4.3	4.6
	B	5.0	3.6	4.3	4.3	4.3	4.3
	C	4.0	4.0	4.0	4.2	4.1	4.2
4.0	A	7.3	6.6	7.0	6.9	6.3	6.6
	B	7.2	6.9	7.0	6.7	6.6	6.6
	C	7.2	6.9	7.0	6.7	6.4	6.6
8.0	A	15.2	14.8	15.0	14.0	12.5	13.2
	B	14.7	15.7	15.2	12.8	13.5	13.2
	C	14.8	15.1	15.0	11.1	11.7	11.4
12.0	A	18.6	19.8	19.2	17.9	16.6	17.2
	B	18.8	18.2	18.5	15.2	14.7	15.0
	C	18.9	19.5	19.2	14.0	14.4	14.2

* Calculations based on the molecular weight of an anhydroglucose unit. This does not account for nitrogen dioxide oxidation which occurred on soluble material; therefore, values are lower than would be calculated if the correct factor were known.

TABLE V

ESTIMATION OF CARBONYL GROUPS BY METHYLHYDROXYLAMINE HYDROCHLORIDE METHOD (24)

Time of Oxidation, Hours	Titration of Liberated Acid from		Carboxyl from CaAc Method	Carbonyl	Kjeldahl Nitrogen* in Washed Residues from		Cu. No.,
	Ca Salt (a) D.S.	Free Acid (b) D.S.	(c) D.S.	(b - c) D.S.	Ca Salt D.S.	Free Acid D.S.	g. Cu./100 g.
0-linters	0.010	0.015	0.004	0.011	0.001	0.001	0.2
0.25	0.045	0.023	0.004	0.0017	0.003	0.009	1.7
0.5	0.066	0.028	0.007	0.021	0.006	0.010	2.2
1.0	0.056	0.049	0.013	0.036	0.010	0.012	2.8
2.0	0.055	0.060	0.028	0.032	0.015	0.019	4.9
4.0	0.065	0.095	0.055	0.040	0.022	0.020	7.4
8.0	0.110	0.132	0.102	0.030	0.047	0.029	9.6
12.0	0.135	0.162	0.122	0.040	0.043	0.033	11.4

* Kjeldahl nitrogen determinations by Analytical Department of the Institute. Values are corrected for nitrogen content in celluronic acids prior to reaction with methylhydroxylamine hydrochloride.

TABLE VI

REPEATED REACTION OF CELLURONIC ACIDS WITH METHYLHYDROXYLAMINE HYDROCHLORIDE

	Time of NO_2 Oxidation, Hours	
	8	12
Carboxyl, D.S.[1]	0.103	0.132
After 1st reaction Carbonyl plus portion of carboxyl, D.S.[2]	0.111	0.149
After 2nd reaction Portion of carboxyl, D.S.[2]	0.047	0.062
After 3rd reaction Portion of carboxyl, D.S.[2]	0.047	0.062

[1]From calcium acetate carboxyl determination.

[2]By titration of liberated hydrochloric acid.

TABLE VII

CHANGES IN CARBOXYL CONTENT OF CELLURONIC ACIDS ON STORAGE

Time of Oxidation, Hours	Original Carboxyl, %	Elapsed Time, Weeks	After Storage Carboxyl, %	Change in Carboxyl, %
2.0	0.77	20	0.66	-0.11
2.0	0.76	20	0.62	-0.14
2.0	0.76	38	0.68	-0.08
4.0	1.56	39	1.70	0.14
4.0	1.57	39	1.42	-0.15
8.0	2.68	17	2.58	-0.10
8.0	2.72	34	2.65	-0.07
12.0	3.36	36	3.60	0.24
12.0	3.32	36	2.93	-0.39
12.0	3.36	35	3.31	-0.05

TABLE VIII

CHANGES IN ALKALINE D.P. OF CELLURONIC ACIDS ON STORAGE

Time of Oxidation, Hours	Original Alkaline D.P.	Elapsed Time, Weeks	After Storage Alkaline D.P.	Changes in Alkaline D.P.
2.0	215	23	175	-40
2.0	237	21	226	-11
2.0	220	34	210	-10
4.0	221	39	187	-34
4.0	243	35	221	-22
8.0	221	10	188	-33
8.0	243	37	170	-73
8.0	230	36	195	-35
12.0	230	38	170	-60
12.0	220	39	155	-65
12.0	201	35	173	-28

TABLE IX

METHYLATION OF A CELLURONIC ACID WITH DIAZOMETHANE IN ETHER AT 0°C.

Reaction Time, Hours	Alkaline D.P.	COOH, %	MeO, %	Degree of Substitution COOH	Ester	Ether	Hydroxyl*
0		3.45	0.20	0.124	0.0	0.010	2.866
1		1.39	2.00	0.050	0.074	0.031	2.845
2		1.50	2.20	0.054	0.070	0.045	2.831
4		1.06	3.16	0.038	0.086	0.079	2.797
8		0.58	3.84	0.021	0.103	0.098	2.778
0	222	3.38	0.20	0.122	0.0	0.010	2.868
48	222	1.08	4.08	0.039	0.083	0.130	2.748

* By difference.

TABLE X

PROPERTY CHANGES ON MODIFICATION OF CELLURONIC ACIDS

Time of Oxidation, Hours	Celluronic Acid			On Chlorous Acid Oxidation				On Sodium Borohydride Reduction			
	COOH, %	Alkaline D.P.	Cu. No.	Yield, %	Changes in COOH, %	Alkaline D.P.	Cu. No.	Yield, %	Changes in COOH, %	Alkaline D.P.	Cu. No
0-linters	0.01	1600	0.2	99.6	--	0	--	100.3	0.04	20	—
0.25	0.10	428	1.7	99.2	0.03	169	-1.5	99.5	-0.05	248	-1.6
0.5	0.20	373	2.7	99.8	0.04	92	-2.1	99.7	-0.14	212	-2.3
1.0	0.31	320	3.4	99.4	0.13	93	-2.4	99.8	-0.21	225	-3.2
2.0	0.62	227	4.9	99.5	0.20	35	-2.7	99.8	-0.02	70	-4.1
4.0	1.44	188	7.4	100.0	0.20	27	-2.9	97.9	-0.30	64	-5.5
8.0	2.58	170	9.6	99.2	0.26	10	-3.2	94.0	-0.37	62	-7.9
12.0	3.06	170	11.4	99.0	0.30	22	-3.5	--	--	--	—
12.0	2.98	187	13.3	--	—	--	--	92.0	-0.21	35	-11.7

TABLE XI

MODIFICATION OF CELLURONIC ACIDS

Time of Oxidation, Hours	Celluronic Acid			Chlorous Acid Oxidized Celluronic Acid				Sodium Borohydride Reduced Celluronic Acid			
	COOH, %	Alkaline D.P.	Cu. No.	Yield, %	COOH, %	Alkaline D.P.	Cu. No.	Yield, %	COOH, %	Alkaline D.P.	Cu. No.
0-linters	0.01	1600	0.2	99.6	--	1600	--	100.3	0.05	1615	—
0.25	0.10	428	1.7	99.2	0.13	597	0.2	99.5	0.05	676	0.1
0.05	0.20	373	2.7	99.8	0.24	465	0.6	99.7	0.06	585	0.4
1.0	0.31	320	3.4	99.4	0.44	413	1.0	98.8	0.10	545	0.2
2.0	0.62	227	4.9	99.5	0.82	262	2.2	98.8	0.60	297	0.8
4.0	1.44	188	7.4	100.0	1.64	215	4.5	97.9	1.14	252	1.9
8.0	2.58	170	9.6	99.2	2.74	180	6.4	94.0	2.21	232	1.7
12.0	3.06	170	11.4	99.0	3.36	192	7.9	--	--	—	--
12.0	2.98	187	13.3	—	--	—	--	92.0	2.77	222	1.6

TABLE XII

EFFECT OF CONCENTRATION OF SODIUM BOROHYDRIDE ON REDUCTION OF A CELLURONIC ACID

Conditions: Reduction in Unbuffered Aqueous Solution for 24 Hours at 20°C.; 100 ml. Solution per Gram Celluronic Acid.

Molar Conc. $NaBH_4$	Intrinsic Viscosity	Alkaline D.P.	D.P. Increase
0	1.23	185	--
0.01	1.35	202	17
0.025	1.46	218	33
0.05	1.58	237	52
0.10	1.54	231	46

TABLE XIII

COMPARISON OF EFFECTS OF SODIUM BOROHYDRIDE AND SODIUM HYDROXIDE SOLUTIONS AT pH 9 ON PROPERTIES OF CELLURONIC ACIDS

	Sodium Borohydride Reduction		Sodium Hydroxide pH Adjustment		Untreated
Sample	A	B	A	B	
pH	9.7	9.7	9.7*	9.7*	
Yield, %	92.6	92.8	95.7	95.0	
COOH, %	2.54		2.62		2.93
Cu. No.	2.4		11.3		13.3
Alkaline D.P.		233		170	155

* Frequent additions of 0.10 N sodium hydroxide were required to maintain pH.

TABLE XIV

ALKALINE EXTRACTION OF CELLURONIC ACIDS
WITH 0.100 N SODIUM HYDROXIDE

Time of Oxidation, Hours	Before Extraction COOH, %	Alkaline D.P.	Cu. No	Yield, %	After Extraction COOH, %	Alkaline D.P.	Cu. No.	Changes on Alkaline Extraction COOH, %	Alkaline D.P.	Cu. No.
0-linters	0.01	1600	0.2	99.2	—	1500	--	--	-100	--
0.5	0.20	373	2.7	98.3	0.12	285	2.5	-0.08	-88	-0.2
1.0	0.31	320	3.4	97.7	0.25	240	3.0	-0.06	-80	-0.4
2.0	0.62	227	--	95.7	0.62	186	--	0	-41	--
2.0	0.68	210	6.4	96.0	0.65	197	4.1	-0.03	-13	-2.3
4.0	1.56	222	--	92.9	1.31	188	--	-0.25	-34	--
4.0	1.42	222	8.5	92.5	1.28	177	4.3	-0.14	-45	-4.2
8.0	2.58	213	--	84.8	1.85	173	--	-0.73	-40	--
8.0	2.65	195	11.0	85.0	1.94	170	5.7	-0.71	-25	-5.3
12.0	2.98	187	--	81.5	2.16	155	--	-0.82	-28	--
12.0	3.31	173	13.2	80.8	2.26	158	5.5	-1.05	-25	-7.7

TABLE XV

ALKALINE EXTRACTION OF CHLOROUS ACID OXIDIZED CELLURONIC ACIDS
WITH 0.100 N SODIUM HYDROXIDE

Time of Oxidation, Hours	Before Extraction Yield, %	COOH, %	Alkaline D.P.	After Extraction Yield, %	COOH, %	Alkaline D.P.	Changes on Alkaline Extraction Total Weight Loss, %	COOH, %	Alkaline D.P.
0-linters	99.6	—	1600						
0.25	99.2	0.13	597						
0.5	99.8	0.24	465	98.5	0.16	399	1.7	-0.08	-66
1.0	99.4	0.44	413	98.4	0.46	324	2.2	0.02	-89
2.0	99.5	0.82	262	96.6	0.98	225	3.9	0.16	-37
4.0	100.0	1.64	215	91.9	1.66	189	8.1	0.02	-26
8.0	99.2	2.74	180	85.8	2.34	174	14.9	-0.40	-6
12.0	99.0	3.36	192	82.2	3.06	155	18.6	-0.30	-37

TABLE XVI

ALKALINE EXTRACTION OF SODIUM BOROHYDRIDE REDUCED CELLURONIC ACIDS WITH 0.100 N SODIUM HYDROXIDE

Time of Oxidation, Hours	Before Extraction			After Extraction			Changes on Alkaline Extraction		
	Yield, %	COOH, %	Alkaline D.P.	Yield, %	COOH, %	Alkaline D.P.	Total Weight Loss, %	COOH, %	Alkaline D.P.
0-linters	100.3	0.05	1620						
0.25	99.5	0.05	676						
0.5	99.7	0.06	585	99.7	0.10	586	0.6	0.04	1
1.0	98.8	0.10	545	99.9	0.26	545	1.3	0.16	0
2.0	98.8	0.60	297	98.8	0.53	294	2.4	-0.07	-3
4.0	97.9	1.14	252	99.8	1.34	232	2.3	0.20	-20
8.0	94.0	2.21	232	98.0	2.15	225	7.9	-0.06	-7
12.0	92.0	2.77	222	98.0	2.74	217	9.8	-0.03	-5

TABLE XVII

DISTRIBUTION OF CARBOXYL GROUPS AND COPPER NUMBER OF CELLURONIC ACIDS BETWEEN ALKALI-SOLUBLE PORTIONS AND ALKALI-EXTRACTED RESIDUES

Time of Oxidation, Hours	Celluronic Acid		Distribution on Alkaline Extraction				
			Extracted Residue			Soluble Portions*	
	COOH, %	Cu. No.	Yield, %	COOH, %	Cu. No.	COOH, %	Cu. No.
0.5	0.20	2.7	98.3	0.12	2.5	4.8	14.2
1.0	0.31	3.4	97.7	0.25	3.0	2.9	20.4
2.0	0.62		95.7	0.62		0.62	
	0.68	6.4	96.0	0.65	4.1	1.4	61.2
4.0	1.56		92.9	1.31		4.8	
	1.42	8.5	92.5	1.28	4.3	3.1	60.2
8.0	2.58		84.8	1.85		6.6	
	2.65	11.0	85.0	1.94	5.7	6.7	41.0
12.0	2.98		81.5	2.16		6.6	
	3.31	13.2	80.8	2.26	5.5	7.7	45.6

*Carboxyl content and copper number of soluble fractions calculated by difference.

TABLE XVIII

ACID HYDROLYSIS OF CELLURONIC ACIDS

Time of Hydrolysis, Hours	O-linters		Time of Nitrogen Dioxide Oxidation, Hours 0.5			1.0			2.0	
	Yield, %	Alkaline D.P.	Yield, %	Alkaline D.P.	Residue COOH, %	Yield, %	Alkaline D.P.	Residue COOH, %	Yield, %	Alkaline D.P.
0		1600		372	0.20		320	0.31		210
0.5	99.0	180	97.2			97.0			97.2	
1.0	96.8	178	95.9	144		95.6	144		95.7	142
2.0	95.7	174	94.5	142		94.1	142		92.7	140
3.0										
4.0	95.7	160	93.0	144		92.8	141		91.0	140
6.0	94.0									
8.0	94.2	162	91.6	142		90.0	141		88.3	140
12.0	92.7*		90.7			89.2			86.9*	
16.0	91.7	159	88.1	142		88.1	141		84.7	141
20.0	91.7		88.4			86.7			85.4	
24.0	90.9	159	87.2	142		85.9	141		83.0	142

* Residues subjected to x-ray diffraction analysis.

TABLE XVIII (Continued)

ACID HYDROLYSIS OF CELLURONIC ACIDS

Time of Hydrolysis, Hours	Time of Nitrogen Dioxide Oxidation, Hours									
	2.0		4.0			8.0			12.0	
	Residue COOH, %	Yield, %	Alkaline D.P.	Residue COOH, %	Yield, %	Alkaline D.P.	Residue COOH, %	Yield, %	Alkaline D.P.	Residue COOH, %
0	0.68		200	1.42		195	2.65		178	3.31
0.5	0.63	97.0		1.38	96.3		2.50			
1.0	0.63	94.9	133	1.33	95.7	126	2.36	92.3	138	3.38
2.0	0.58	92.9	130	1.32	90.3	123	2.37			
3.0					89.9		2.30			
4.0	0.55	90.3	130	1.24	88.6	129	2.29	88.4	124	3.20
6.0										
8.0	0.52	86.6	130	1.18	86.1	126	2.33	85.7	124	2.90
12.0	0.60	83.8		1.18	80.6		2.18	83.8*		2.86
16.0	0.58	83.1	130	1.14	83.0	126	2.19	80.5	124	2.76
20.0	0.52	84.1		1.12	83.3		2.10			
24.0	0.51	84.2	132	1.04	81.5	130	1.99	80.8	124	2.68

* Residues subjected to x-ray diffraction analysis.

TABLE XIX

COMPARATIVE D.P. OF CELLURONIC ACIDS

Time of Oxidation, Hours	Celluronic Acid Alkaline D.P.	$NaBH_4$ Reduced Celluronic Acid Alkaline D.P.	Nitrated Celluronic Acid N., %	Nitrated Celluronic Acid D.P.*	Nitrated $NaBH_4$ Reduced Celluronic Acid N., %	Nitrated $NaBH_4$ Reduced Celluronic Acid D.P.*
0-linters	1600	1615	13.7	1232	13.0	1270
0-linters			12.5	S		
0.25	428	676	13.0	604	12.4	630
0.5	373	585	13.1	562	13.0	576
1.0	320	545	12.4	PS	12.9	472
2.0	227	297	13.7	MS	13.7	414
4.0	188	252		MS	11.7	251
8.0	170	232		MS	12.8	324
12.0	187	222	12.6	MS	12.6	378

S Soluble in acetone and ethyl lactate
PS Partially soluble, gelatinous mass formed in acetone and ethyl lactate
MS Moderately swollen, fibers transparent but retain individual identity

* D.P. of nitrated materials determined from ethyl lactate solution viscosities.

SOLUBILITY OF CELLURONIC ACIDS AND MODIFIED CELLURONIC ACIDS IN NONALKALINE SOLVENTS

Solubility tests were conducted at room temperature by placing a small portion of the sample in a test tube to which the solvent was then added. A piece of glass rod was also added to aid agitation during occasional periods of shaking. After 3 hours, the degree of solubility was qualitatively estimated by observing the swelling of the sample. Continuation of the test for more than 3 hours did not produce a noticeable change in the degree of swelling.

Celluronic acids and modified celluronic acids prepared during characterization studies were found to be insoluble in the following solvents:

Ethyl acetate
Ethylene chlorohydrin
Ethylene dichloride
Diacetone alcohol
2-Nitropropane
Methyl ethyl ketone
Pyridine
Formamide
Dimethylformamide

Of these solvents, only formamide dissolved Oxycell--a high carboxyl surgical gauze marketed by Parke Davis.

Phosphoric acid, 85.4%, caused a high degree of swelling of the celluronic acids and modified celluronic acids, but complete solution was not attained. Unoxidized cotton linters dissolved in phosphoric acid.

The nitrates derived from:-

Linters
Celluronic acids
Chlorous acid-oxidized celluronic acids
Sodium borohydride-reduced celluronic acids
Diazomethane-treated celluronic acids
Methylhydroxylamine-reacted celluronic acids

were insoluble in:

Ethylene chlorohydrin
Ethylene dichloride
Ethylene glycol
Glacial acetic acid
Formamide

The same nitrates were slightly swollen by:

Triethylene glycol
Dibutyl phthalate

These nitrates were moderately swollen (fibers became transparent but retained identity) in:

Diacetone alcohol

and formed a gelatinous mass in:

Pyridine
Nitrobenzene
n-propyl acetate

The nitrates of

Linters
Sodium borohydride-reduced celluronic acids
Methylhydroxylamine-reacted celluronic acids

formed gelatinous masses, while the nitrates of

Celluronic acids
Chlorous acid-oxidized celluronic acids
Diazomethane-reacted celluronic acids

were only moderately swollen in:

Ethyl acetate
Methyl ethyl ketone
2-Nitropropane
Nitroethane

Complete solubility of nitrates of:

Linters
Reduced celluronic acids

and swelling to a gelatinous mass of the nitrates of:

Chlorous acid-oxidized celluronic acids
Diazomethane-reacted celluronic acids

occurred in:

Ethyl lactate	Dimethylformamide
Butyl acetate	Tetrahydrofuran
Methyl cellosolve acetate	Acetone

From the solubility tests, it was concluded that the normal solvents for cellulose nitrates, i.e., ethyl lactate and acetone, would be best in D.P. determination on nitrated linters and nitrated-reduced celluronic acids.

EXPERIMENTAL METHODS

PREPARATION OF COTTON LINTERS

The cotton linters as received contained a small number of loose knots of fiber which were easily dispersed by agitation of the slush stock. Therefore, approximately 600-gram batches of linters in 8 liters of water were dispersed with a Williams disintegrator for 5 minutes.

In approximately 200-gram portions, the dispersed linters were transferred to a 16-cm. diameter fritted glass (C) filter funnel and the excess water removed by suction. The linters were then solvent-exchange dried with acetone and ether. The solvent sequence used consisted of three 2-liter portions of acetone and three 1-liter portions of anhydrous ether. Each portion of acetone and ether was used progressively on three batches of linters, with the final exchange with each solvent on every batch consisting of fresh solvent.

After a final ether exchange, the linters were spread on a screen until most of the residual ether evaporated. While still damp with ether, 4 to 5-gram batches of linters were fluffed in a Waring Blender. After fluffing, the linters were air dried, randomized, and stored in polyethylene bags. The linters prepared in this manner were in a highly fluffed form, suitable for insuring rapid penetration of reagents to all fibers.

CALCIUM ACETATE CARBOXYL DETERMINATION

The carboxyl contents of the celluronic acids were estimated by the

calcium acetate method (2,4,10,24,50). Duplicate samples of celluronic acids weighing 0.300 to 1.000 gram were weighed into glass-stoppered Erlenmeyer flasks. The weight of the sample was adjusted to compensate for variations in the equilibrium pH. Although an equilibrium pH of 6.3 to 6.7 has been recommended (24), strict adherence has been found unnecessary (10). To each sample, 75.0 ml. of ca., 0.25 N calcium acetate solution was added, and the reaction was permitted to proceed for a minimum of 15 hours (24) at 20°C. The samples were then filtered on a dry filter and a 50.0-ml. aliquot was titrated with 0.010 N sodium hydroxide to an endpoint pH of 8.3, as determined with a pH meter.

ASH

The ash contents of the cotton linters and celluronic acids were determined by igniting samples in a muffle furnace at 500°C. for 4 hours.

SPECTROGRAPHIC ANALYSIS OF ASH

The Physics Department of the Institute determined the constituents of the ash by spectrographic analysis. The ash was mixed with spectrographically pure graphite, formed into a pellet and placed in a specially prepared carbon electrode. The arc was excited with alternating current. Exposure on a type 2-O plate was made through a 20-micron slit on the Bausch and Lomb medium quartz spectrograph.

CHLOROUS ACID OXIDATION

The conditions of Meesook and Purves (24) were used for chlorous acid oxidations of linters and celluronic acids, except that the products were dried with acetone and ether.

SODIUM BOROHYDRIDE REDUCTION

The reduction of linters and celluronic acids was based on the procedure of Head (38,39). An unbuffered solution of sodium borohydride in water was used at 20°C. for 24 hours. For each gram of cellulose or celluronic acid, 100 ml. of sodium borohydride solution was used. After an investigation of the effect of concentration (Table XII), a 0.05 molar solution of borohydride was adopted.

DIAZOMETHANE TREATMENT

An ethereal solution of diazomethane was used for the methylation of celluronic acids according to the procedure of Head (36). The synthesis of diazomethane (51) from nitrosomethyl urea (52) is described by Arndt.

METHOXYL CONTENT

The methoxyl content of the esterified celluronic acids was estimated by Institute Method 18 (53).

SOLUBILITY IN 0.100 N SODIUM HYDROXIDE

Institute Method 421 (54) for the determination of alpha-cellulose was followed for estimating the solubility of celluronic acids, except that the sodium hydroxide was 0.100 N.

POTASSIUM DICHROMATE OXIDATION

The cellulosic materials removed by alkaline extraction were estimated by wet oxidation with potassium dichromate. Institute Method

425 (55) was followed, except 5.0 ml. of 0.20 N potassium dichromate and approximately 0.025 N ferrous ammonium sulfate were used.

ALKALINE D.P.

The technique of Browning, Sell, and Abel (56) was used for the preparation of solutions containing accurately weighed samples of approximately 0.050, 0.100, 0.200, and 0.250 grams of cellulose or celluronic acid per 100 ml. of 0.5 molar cupriethylenediamine. The precautions of removing residual oxygen from prepurified nitrogen, protecting the serum bottles from light, and flushing the viscometers with nitrogen were not observed. The viscosities of the solutions were measured in Cannon-Fenske viscometers at 20°C.

The intrinsic viscosity of each material was obtained by an extrapolation to zero concentration of a plot of the logarithm of reduced viscosity was multiplied by the factor 150 (29).

NONALKALINE D.P. (Ethyl Lactate)

The nitrated cellulose, celluronic acid, or modified celluronic acid was dissolved in ethyl lactate containing 1.5% water by volume. Aliquots were then diluted to concentrations of approximately 0.050, 0.100, 0.150, and 0.200 gram per 100 ml. of ethyl lactate solvent. The exact concentration of each solution was known. Viscosities of the solutions were measured in Cannon-Fenske viscometers at 20°C. Duplicate determinations were made on each nitrated sample. The intrinsic viscosity was found as for alkaline D.P. The D.P. of the nitrated samples was calculated by multiplying the intrinsic viscosity by the factor 90 (29).

HYDROLYSIS IN 1.0 N HYDROCHLORIC ACID

Two-gram samples of cotton linters and celluronic acids were hydrolyzed with 1.000 N hydrochloric acid in a boiling water bath. The hydrolysis experiments were conducted in 100-ml. flasks under reflux. For each 2-gram sample, 75.0 ml. of standard acid was used. After the desired hydrolysis period, the samples were filtered onto tared fritted glass (C) filter crucibles, washed with hot water until chloride free, and dried by solvent exchange with acetone and ether. The residual ether was removed by drying at 50°C. under vacuum until the residues were odorless. After cooling, the weight of the residue was obtained.

PREPARATION OF SAMPLES FOR X-RAY DIFFRACTION ANALYSIS

Five grams of cotton linters were hydrolyzed for 12 hours in 1.000 N hydrochloric acid. A portion of the hydrolyzed linters were used for an example of Cellulose I, while the remainder were mercerized with 17.5% sodium hydroxide for 1 hour at 20°C. for an example of Cellulose II. The residues from 12-hour hydrolysis of celluronic acids which had been obtained by 2-hour and 12-hour oxidations with 6% nitrogen dioxide in carbon tetrachloride constituted the other samples. The four samples were then submitted to Professor G. L. Clark for preparation of the x-ray diffraction charts.

NAPHTHORESORCINOL TEST FOR URONIC ACIDS

The naphthoresorcinol test for uronic acids of Tollens and Rorive (48) was used for the tentative identification of glucuronic acid. A few milligrams of the sample was dissolved in 12% hydrochloric acid, an

equal weight of naphthoresorcinol in ethanol was added and the mixture was heated for 15 minutes in a boiling water bath. After cooling, the solution was extracted with 50:50 benzene:ethyl ether. A blue to purple color in the organic layer indicated the presence of uronic acid.

COPPER NUMBER

The copper number was determined by the Luff-Schoorl method (30), except that the heating and boiling were accomplished by placing the reaction flask on a hot plate for 10 minutes. Copper number was calculated as grams of copper reduced per 100 grams of sample.

METHYLHYDROXYLAMINE HYDROCHLORIDE CARBONYL DETERMINATION

A 0.15 N solution of methylhydroxylamine hydrochloride which had been buffered to pH 4.6 with sodium hydroxide was reacted with 0.2 to 1.0-gram samples for 24 hours at 20°C. The procedure was based on the method of Meesook and Purves (24).

NITRATION

Cotton linters, celluronic acids, and modified celluronic acids were nitrated according to the method of Mitchell (57).

NITROGEN ANALYSES

The nitrogen contents of methylhydroxylamine hydrochloride-reacted celluronic acids were determined by the Analytical Department of the Institute using a Kjeldahl method (31).

The nitrogen contents of the celluronic acids were determined by the

Dumas method according to Institute Method 708 (27). These analyses were also conducted by the Analytical Department of the Institute.

The nitrogen contents of nitrated celluronic acids were determined by the method of Shaefer and Becker (58).

PRIMARY HYDROXYL GROUPS ON CRYSTALLITE SURFACES

The number of primary hydroxyl groups which are exposed and can be oxidized readily on the surfaces of crystallites can be estimated from the dimensions of crystallites (59) and the cellulose unit (cellobiose) (60). In the following calculation, any hydroxyl groups that can react on the ends of the crystallites have been disregarded. The dimensions are as follows:

	Width	Depth	Length
Crystallite dimensions, angstroms	50	100	5-600
Unit cell dimensions, angstroms	7.9	8.35	10.3
Unit cells along each dimension of the crystallites	6	12	50
Cellobiose units along each dimension of the crystallites	7	13	50

From these values, it is possible to calculate the number of cellobiose units on the surface and the total number present in a crystallite.

Surface units = $(2 \times 7 + 2 \times 13 - 4) \times 50$ = 1800

Total units = $(7 \times 13 \times 50)$ = 4550

Cellobiose units on surface, % = 39.6

For each cellobiose unit on the surface, one primary hydroxyl group

is exposed for reaction; therefore, 19.8% of the total hydroxyl groups can be oxidized to carboxyl groups before other than surface hydroxyls enter into reaction.